Aus dem Departement Biologie, Institut für Ökologie und Evolution
Universität Freiburg (Schweiz)

Land use, soil nutrient availability and conservation of biodiversity on mountain grasslands

INAUGURAL-DISSERTATION

zur Erlangung der Würde eines *Doctor rerum naturalium*
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Freiburg in der Schweiz

vorgelegt von

Thomas Spiegelberger

aus
Deutschland

Dissertation Nr. 1512
Eigenverlag
2006

Von der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Freiburg in der Schweiz angenommen, auf Antrag von Dr. Urs Schaffner, Prof. Dr. Diethart Matthies und Prof. Dr. Fritz Müller.

Freiburg, den 03.04.2006

Der Dissertationsleiter:

Prof. Dr. Heinz Müller-Schärer

Der Dekan:

Prof. Dr. Marco Celio

Contents

Abstract

Biodiversity decreases world-wide in an unprecedented rate. In Europe many traditionally managed mountain grasslands are still species-rich, but a shift from the traditional, extensive land use to a more intensive use of well accessible sites and to a gradual abandonment of remote sites is threatening these habitats. In both cases, nutrient availability may change due to fertiliser or to litter accumulation. The general aim of this thesis was to further our comprehension of the importance and the results of changes in land use and in soil nutrient availability on soil chemistry, belowground microorganisms and vegetation of mountain grasslands with regard to an unpalatable weed (*Veratrum album*) and to mitigate negative developments due to land use change of mountain grasslands.

In five regions of the Alps we showed that mean species richness was lower in intensively grazed, fertilised pastures than in traditionally, extensively grazed or in abandoned pastures. Species composition of abandoned pastures differed from that of the other management types. Species richness at the 1 m² scale was negatively related to soil nitrate and influenced by cover of *V. album*, depending on land use: species richness and cover of *V. album* were negatively correlated in abandoned pastures, but positively correlated in fertilised grasslands. At the 1000 m² scale, species richness was negatively influenced by fertilisation. These results indicate that at small scales species richness is determined by competition for light and by positive and negative interactions with unpalatable plants. In contrast, species richness at the large scale appears to be mainly influenced by land use.

Changes in the management of grasslands can have very long lasting effects as harsh environmental conditions at high altitudes constrain many factors important for biodiversity. Results from a controlled, large-scale field experiment in a sub-alpine grassland show that 2-4 years of liming (40 g m^{-2} y^{-1}) still significantly affected the vegetation composition and the soil microbial community nearly 70 years after the treatments were imposed, whereas NPK fertilization (8 g m^{-2} y^{-1}) only marginally affected vegetation composition. The higher exchangeable Ca^{2+}-ion content and pH on limed plots together with plant species and PLFAs typical for high pH suggested that the long-lasting effects of liming on the above- and below-ground communities were mediated through changes in soil pH. These results indicate that the resilience of mountain ecosystems may be particularly low to perturbations that substantially alter soil pH or other key determinants of belowground processes.

One possible countermeasure against augmented plant nutrient availability is carbon (C) addition to the soil. Such a treatment has been found to increase soil microbial populations that take up nitrogen (N) and make it temporally unavailable for plants. In turn, lower soil inorganic N availability may favour slow-growing species typical for species-rich mountain grasslands. Results of a 3-year, multi-site field study showed that cover of grasses was significantly lower on sawdust amended plots, while forbs were not significantly influenced. Total biomass of all plants except *V. album* was lower on the C-amended plots. No effect of C-addition was found on soil inorganic nutrient pools. We conclude that sawdust addition can decrease productivity of both grazed and ungrazed mountain grassland. It is a cheap and simple tool to reduce especially the cover and above-ground biomass of grasses, which may reduce competition for subdominant species.

However, in cold climate ecosystems plants can also access organic N with the aid of arbuscular mycorrhizal fungi (AMF). Therefore a more mechanistic understanding of the effects of nutrient manipulations is needed. In a bioassay we showed that C decreased and N or phosphorus (P) fertilisation increased biomass productivity of the phytometer species *Festuca rubra* and *Poa alpina*. While *P. alpina* (tended to have lower AMF-colonisation rates) showed no reaction to application, *F. rubra* (high AMF-colonisation rates) produced about 20 % (N) and about 30 % (C) less biomass when the fungicide benomyl was applied. As soil micro-organisms are at least temporally better competitors for plant available nutrients than plants, C addition may decrease primarily the above-ground biomass of plants having low AMF-colonisation rates.

The results of these studies suggest that the maintenance of the traditional land use is crucial for conservation of plant species richness of mountain grasslands as both intensification and gradual extensification until complete abandonment changed species composition and reduced plant species diversity. Already short-term soil amendments can have long lasting effects and can substantially change the vegetation composition. A promising tool to reduce N availability to plants is C-addition. It reduces over-proportionally the productivity of less AMF-colonized plants and may therefore favour species richness.

Zusammenfassung

Weltweit geht der Artenreichtum mit nie zuvor gesehener Geschwindigkeit zurück. In Europa sind viele traditionell bewirtschafteten Berggebiete noch artenreich, aber eine Verschiebung von der traditionellen, extensiven Nutzung zu einer intensiveren Nutzung von gut zugänglichen Gebieten und eine schrittweise Aufgabe von abgelegenen Flächen bedroht diese Habitate. In beiden Fälle könnte sich die Nährstoffverfügbarkeit durch Düngung oder durch die Ansammlung von verrottendem Pflanzenmaterial verändern. Ziel dieser Arbeit war es, die Bedeutung von Landnutzungs-veränderungen von Berg-Grünland im Zusammenhang mit einem giftigen Unkraut (Germer, *Veratrum album*) auf die Bodennährstoffverfügbarkeit, die unterirdischen Mikroorganismen und die Vegetation zu untersuchen und negative Entwicklungen zu reduzieren.

In fünf Regionen in den Alpen zeigten wir, dass die mittlere Artenzahl in intensiv beweideten, gedüngten Weiden niedriger war im Vergleich zu traditionell bewirtschaften, extensiv genutzten Weiden. Die Vegetationszusammensetzung aufgegebener Weiden unterschied sich von der der zwei anderen Nutzungstypen. Der Artenreichtum auf 1 m² war negativ mit dem Bodennitratgehalt korreliert und - abhängig von der Landnutzung - von der Deckung des Germers beeinflusst: Artenreichtum und Deckung des Germers waren auf aufgegebenen Weiden negativ und auf gedüngten Weiden positiv korreliert. Auf der 1000 m²-Ebene war der Artenreichtum negativ von der Düngung beeinflusst. Diese Ergebnisse zeigen, dass kleinräumig der Artenreichtum durch Lichtkonkurrenz und von positiven und negativen Interaktionen mit ungenießbaren Pflanzen bestimmt wird. Großräumig

jedoch, ist der Artenreichtum hauptsächlich von der Landnutzung beeinflusst.

Veränderungen in der Nutzung von Grünland können in hohen Lagen sehr lang andauernde Effekte haben, da harsche Umweltbedingungen viele für die Biodiversität bedeutende Fakoren beeinflussen. Ergebnisse eines kontrollierten, großräumigen Feldexperiments in einem subalpinen Grünland zeigen, dass durch 2–4 Jahre Kalkung (40 g m^{-2} y^{-1}) auch 70 Jahre nach dem Ende der Zugaben noch signifikant die Zusammensetzung der Vegetation und der Boden-Mikroorganismen bestimmt wurde, während NPK-Düngung (8 g m^{-2} y^{-1}) nur noch schwach die Vegetations-zusammensetzung beeinflusste. Der höhere Anteil der austauschbaren Ca^{2+}-Ionen und des pH-Werts auf gekalkten Flächen, zusammen mit Pflanzenarten und PLFAs typisch für hohe pH-Werte lässt vermuten, dass der lang anhaltende Effekt der Kalkung auf oberirdische- und unterirdischen Artenzusammensetzung durch den pH-Wert bestimmt wird. Die Studie zeigt, dass die Widerstands-fähigkeit von Berg-Grünland insbesondere gegenüber Veränderung, die nachhaltig den Boden-pH-Wert oder anderer Schlüsselmerkmale von Bodenprozessen verändern, niedrig ist.

Eine mögliche Maßnahme gegen erhöhte Pflanzennährstoff-verfügbarkeit ist die Zugabe von Kohlenstoff (C) zum Boden. Solch eine Zugabe führt zu einer Erhöhung der Menge der Boden-mikroorganismen, die Stickstoff (N) aufnehmen und ihn zeitlich für die Pflanzen unverfügbar machen. Niedrige anorganische N-Werte im Boden fördern vermutlich langsam wachsende Pflanzen typisch für artenreiches Berg-Grünland. Die Ergebnisse einer dreijährigen, an mehreren Orten durchgeführten Feldstudie zeigen, dass die Grassdeckung signifikant niedriger auf den C-gedüngten Flächen war,

wohingegen die Deckung der Kräuter nicht signifikant beeinflusst wurde. Die totale Biomasse aller Pflanzen außer *V. album* war niedriger auf der C-gedüngten Fläche. Kein Effekt der C- Zugabe wurde auf den anorganischen Nährstoffpool festgestellt. Wir fassen zusammen, dass Sägemehl-Zugabe die Produktivität von beweideten und unbeweideten Berg-Grünland verringern kann. Es is eine billige und einfache Möglichkeit, um insbesondere die Deckung und die oberirdische Biomasse von Gräsern zu verringern, was in der Folge die Konkurrenz für untergeordnete Arten reduzieren könnte.

In Ökosystemen in kalten Klimazonen habe die Pflanzen jedoch mit der Hilfe von arbuscularen Mykorrhiza-Pilzen (AMF) Zugriff auf organisches N. Deshalb ist ein besseres Verständis der Mechanismen nötig, die diesen Nährstoffveränder-ungen zugrunde liegenden. In einem Bioassy zeigten wir, dass C die Biomasse der zwei Phytometer-Arten *Festuca rubra* und *Poa alpina* reduzierte und N und Phosphor (P) diese erhöhte. Während *P. alpina* (geringer AMF-Kolonisierungsrate im Vergleich zu *F. rubra*) nicht durch die Anwendung eines Fungizids beeinflusst wurde, produzierte *F. rubra* 20% (N) bzw. 30% (C) weniger Biomasse, wenn das Fungizide Benomyl gespritzt wurde. Da die Boden-Mikroorganismen zumindest zeitweise besser um pflanzenverfügbare Nährstoffe konkurrieren können, werden in erster Linie Pflanzen mit geringer AMF-Kolonisierungsrate von der C-Zugabe betroffen sein.

Diese Studien zeigen, dass die Beibehaltung der traditionellen Landnutzung entscheidend für die Erhaltung des Pflanzenreichtums des Berg-Grünland ist, da sowohl Intensivierung als auch Extensivierung die Artenzusammensetzung verändern und den Artenreichtum verringern. Schon kurzfristige Veränderungen können nachhaltige Auswirkungen auf die Artenzusammensetzung haben.

Eine vielversprechende Möglichkeit das pflanzenverfügbare N zu verringern, ist C-Zugabe. Sie reduziert überproportional die Produktivität von wenig AMF-kolonisierten Pflanzen und könnte daher den Artenreichtum fördern.

Introduction

Biodiversity

Globally, biodiversity is changing at an unprecedented rate as a complex response to several human-induced changes (Vitousek 1994, Hooper et al. 2005). These changes in biodiversity cause concern for ethical, economical, ecological, and aesthetic reasons, but they also have a strong potential to alter ecosystem services such as the prevention of soil erosion and maintenance of hydrologic cycles, and ecosystem goods, like tourism and recreation. Beyond the ecosystem services, biodiversity influences many ecosystems properties such as productivity, decomposition rates, nutrient cycling, and resistance and resilience to perturbations (Loreau et al. 2001). Moreover, a high biodiversity is seen as an insurance against a decline in ecosystem services, and should therefore be preserved (Yachi and Loreau 1999).

Mountain grasslands

Mountain grasslands below the treeline are in most cases semi-natural ecosystems which were created by man through logging. Mountain grasslands are found in the montane and subalpine zones between 800 m asl and the current treeline (around 1800 m asl for the Northern Alps). In Switzerland, mountain pastures occupy 940.000 ha, i.e. almost one fourth of the total land area.

Traditionally mountain grasslands are grazed by heifers or dairy cattle during the summer months from the end of May until end of September, and do not receive fertiliser. They are characterized by a large number of different microsites (Austrheim and Eriksson 2001, Erschbamer et al. 2003) originating from small-scale variation in topography and increased through grazing that multiplies the number

of microhabitats through spatially heterogeneous defoliation, trampling, wallowing and faecal deposition (WallisDeVries et al. 1998).

Many traditionally managed mountain grasslands are still more species-rich than the surrounding lowlands (MacDonald et al. 2000) and start to function increasingly as refuge for species that once were common throughout Europe (Nösberger et al. 1994). However, changes in land use and nutrient enrichment have been found to become beside climatic changes the two most important determinants of biodiversity modifications of mountain ecosystems (Sala et al. 2000).

Land use change in mountain grasslands

In the recent past, mountain grasslands have started undergoing changes in land use. Currently, a shift from traditionally used, extensively grazed pastures to a more intensive use of well accessible sites or to a gradual abandonment of remote sites is taking place in the European Alps (Tasser and Tappeiner 2002). One reason for this change is the improved productivity of lowland grasslands due to fertilisers less expensive than in the past and the easier availability of additional fodder (concentrated feeding stuff), which reduced food shortage. In consequence, the importance of mountain grasslands as summer pastures has decreased. Changes in disturbance regimes (here grazing) often have dramatic effects on community composition and structure, especially in natural or semi-natural systems with high diversity and long disturbance histories (Milchunas et al. 1988).

Both, intensification and extensification of mountain grasslands alter biodiversity (Austrheim and Eriksson 2001, Fischer and Wipf 2002, Dullinger et al. 2003, Zechmeister et al. 2003), but effects may vary depending on spatial scale. Decreasing cattle density may reduce small-scale heterogeneity and may shift competition between plant species, while changes in management of whole grasslands may alter the vegetation composition and species richness on large scales. However, the patterns how biodiversity and land use of mountain grasslands are related on different spatial scales remain for the moment unexplored.

Nutrient enrichment

The availability of nutrients, in particular of nitrogen (N), is one of the most important determinants of vegetation composition and N is the limiting resource for plant growth in many ecosystems (Vitousek 1982). Theory predicts that above a certain level of primary productivity, local species diversity declines as productivity increases. This has be demonstrated by theoretical work and numerous field studies (Gough et al. 2000, Gross et al. 2000, Suding et al. 2005).

In Europe, critical loads for grasslands are exceeded in many parts (Holland et al. 2005). Although nitrogen deposition rates are still low in mountain areas compared to lowland parts, they are high with regard to rates of internal N cycling. Cold ecosystems are characterized by low N mineralization rates and low biotic uptake (Schmidt et al. 1999, Weintraub and Schimel 2003). Thus, the potential for impacts of anthropogenic increased N enrichment on mountain ecosystems is relatively high. The extensive agricultural use with nutrient removal through grazing and no or only very low nutrient

input for many centuries has generally increased the nutrient poor status of mountain grasslands. Most of the typical mountain grassland plant species are well adapted to these conditions and can only compete successfully on soils with low nitrogen content (Aerts 1999). In consequence, one would expect that these habitats will be changed by the increased anthropogenic N input. Therefore, methods need to be developed to minimize the potential loss of biodiversity or to favour the restoration of nitrogen enriched communities.

Unpalatable weeds

Unpalatable weeds may play an important role at the interface between land use and nutrient enrichment of mountain grasslands. High weed densities, in particular of weeds toxic to cattle, reduce the fodder quality and thus the economic value of a grassland. In consequence, grasslands with a high weed density could be more prone to a gradually abandonment compared to grasslands with a lower percentage of weeds. On the other hand, when grazing by cattle is intensified, unpalatable weeds may increase in density as they are at least partially protected against grazing. Unpalatable weeds can therefore be seen as a starting point of land use changes in the case of decreasing grazing pressure, but also as a result of land use changes when grazing intensity is increased.

Many unpalatable weeds on mountain grasslands are tall, unpalatable forbs which have large below-ground organs (e.g. *Cirsium eriophorum* (L.) Scop., *Gentiana lutea* L., *Rumex alpinus* L., *Senecio alpinus* (L.) Scop. and *Veratrum album L.*). Because of their tall growth, they may negatively affect species richness at small scales

simply due to competition for space (Crawley and Harral 2001). Some of those species can reach high densities on under-exploited pastures or on pastures undergoing a process of gradual abandonment and are therefore regarded as extensification weeds. One of the most important extensification weeds is the highly toxic monocot *V. album*. It is native to Europe and Asia and has attained pest status in France, Switzerland, Italy, Austria and Slovenia (FAO, unpublished report).

Outline of the thesis

This dissertation was carried out in the framework of the Swiss Priority Program (NSP 48) “Landscapes and Habitats of the Alps”, a research project of the National Science Foundation of Switzerland. The general aim of this thesis was to further our comprehension of the importance and the results of changes in land use and in soil nutrient availability on soil chemistry, belowground microorganisms and vegetation of mountain grasslands in the context of unpalatable weeds. Therefore, I chose *V. album* as a model species for long-lived, tall, and unpalatable weeds common on mountain grasslands. Further, I wanted to understand the mechanism behind species richness of mountain grasslands and to mitigate negative developments due to land use change.

A correlative field study on the effects of different types of land use on plant species richness and on the abundance of undesirable plant species was conducted, in which I investigated at different spatial scales ranging from 1 m² – 1000 m² the underlying mechanism influencing species richness on mountain grasslands (chapter 2). Long-term effects and resilience to nutrient manipulations were studied in a subalpine grassland next to the treeline, where lime and NPK-

fertiliser was applied for the last time 70 years ago (chapter 3). In a multi-site approach, sawdust application to native, perennial vegetation was tested as a low cost method to decrease plant available nutrients and to reduce above-ground biomass and cover of grasses and *V. album* susceptible to negatively influence species richness (chapter 4). Based on the last experiment I tried to shed some light on the role of soil microorganisms, in particular arbuscular mycorrhizae, in relation to altered soil nutrient status (chapter 5). In chapter 6, I summarise and combine the results of these studies in order to develop general conclusions about the effects of land use and soil nutrient changes on mountain grassland diversity.

References

Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. - J. Exp. Bot. 50: 29-37.

Austrheim, G. and Eriksson, O. 2001. Plant species diversity and grazing in the Scandinavian mountains - patterns and processes at different spatial scales. - Ecography 24: 683-695.

Crawley, M. J. and Harral, J. E. 2001. Scale dependence in plant biodiversity. - Science 291: 864-868.

Dullinger, S., Dirnböck, T., Greimler, J. and Grabherr, G. 2003. A resampling approach for evaluating effects of pasture abandonment on subalpine plant species diversity. - J. Veg. Sci. 14: 243-252.

Erschbamer, B., Virtanen, R. and Nagy, L. 2003. The impacts of vertebrate grazers on vegetation in Europe high mountains. - In: Nagy, L., Grabherr, G., Körner, C. and Thompson, D. B. A. (eds.), Alpine Biodiversity in Europe. Springer, pp. 377-396.

Fischer, M. and Wipf, S. 2002. Effect of low-intensity grazing on the species-rich vegetation of traditionally mown subalpine meadow. - Biol. Conserv. 104: 1-11.

Gough, L., Osenberg, C. W., Gross, K. L. and Collins, S. L. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. - Oikos 89: 428-439.

Gross, K. L., Willig, M. R., Gough, L., Inouye, R. and Cox, S. B. 2000. Patterns of species density and productivity at different spatial scales in herbaceous plant communities. - Oikos 89: 417-427.

Holland, E. A., Braswell, B. H., Sulzman, J. and Lamarque, J. F. 2005. Nitrogen deposition onto the United States and western Europe: Synthesis of observations and models. - Ecol. Appl. 15: 38-57.

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. - Ecol. Monogr. 75: 3-35.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., Hooper, D. U., Huston, M. A., Raffaelli, D., Schmid, B., Tilman, D. and Wardle, D. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. - Science 294: 804-808.

MacDonald, D., Crabtree, J. R., Wiesinger, G., Dax, T., Stamou, N., Fleury, P., Gutierrez Lazapita, J. and Gibon, A. 2000. Agriculture abandonment in mountain areas of Europe: Environmental consequences and policy response. - J. Environ. Manage. 59: 47-69.

Milchunas, D. G., Sala, O. E. and Lauenroth, W. K. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. - Am. Nat. 132: 87-106.

Nösberger, J., Lehmann, J., Jeangros, B., Dietl, W., Kessler, P., Bassetti, P. and Mitchley, J. 1994. Grassland production systems and nature conservation. - In: T'Mannetje, P. and Frame, J. (eds.), Grassland and Society. Proceedings of the 15th General Meeting of the European Grassland Federation, pp. 255-265.

Sala, O. E., Chapin III, F. S., Armesto, J. J., Berlow, E., Bloomfeld, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A. P., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, L., Sykes, M. T., Walker, B. H., Walker, M. and Wall, D. H. 2000. Global biodiversity scenarios for the year 2100. - Science 287: 1770-1774.

Schmidt, I. K., Jonasson, S. and Michelsen, A. 1999. Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. - Applied Soil Ecology 11: 147-160.

Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., Gross, K. L., Milchunas, D. G. and Pennings, S. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. - Proc. Nat. Acad. Sci. USA 102: 4387-4392.

Tasser, E. and Tappeiner, U. 2002. Impact of land use changes on mountain vegetation. - Appl. Veg. Sci. 5: 173-184.

Vitousek, P. M. 1982. Nutrient cycling and nutrient use efficiency. - Am. Nat. 119: 553-572.

Vitousek, P. M. 1994. Beyond global warming: ecology and global change. - Ecology 75: 1861-1876.

WallisDeVries, M. F., Bakker, J. P. and Van Wieren, S. F. (eds.). 1998. Grazing and Conservation Management. - Kluwer Academic Publishers.

Weintraub, M. N. and Schimel, J. P. 2003. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in arctic tundra soils. - Ecosystems 6: 129-143.

Yachi, S. and Loreau, M. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. - Proc. Nat. Acad. Sci. USA 96: 1463-1468.

Zechmeister, H. G., Schmitzberger, I., Steurer, B., Peterseil, J. and Wrbka, T. 2003. The influence of land-use practices and economics on plant species richness in meadows. - Biol. Conserv. 114: 165-177.

Scale-dependent effects of land use on plant species richness of mountain grassland in the European Alps

Thomas Spiegelberger, Diethart Matthies, Heinz Müller-Schärer and Urs Schaffner

Paper published in *Ecography*, **29**, pp. 541-548 (2006)

Abstract

Traditionally managed mountain grasslands in the Alps are species-rich ecosystems that developed during centuries of livestock grazing. However, changes in land use including fertilisation of well accessible pastures and gradual abandonment of remote sites are increasingly threatening this diversity. In five regions of the Swiss and French Alps we assessed the relationship between land use, soil resource availability, cover of the unpalatable species *Veratrum album*, species richness and vegetation composition of mountain grasslands across four spatial scales ranging from 1 to 1000 m².

Mean species richness and the increase in the number of species with increasing area were lower in intensively grazed, fertilised pastures than in traditional pastures or in abandoned pastures. Species composition of abandoned pastures differed from that of the other management types. Plant species richness was influenced by different factors at different spatial scales. At the 1 m² scale, plant species richness was negatively related to soil nitrate and influenced by the cover of *V. album*, depending on land use: species richness and cover of *V. album* were negatively correlated in abandoned pastures, but positively correlated in fertilised grasslands. At the 1000 m² scale, a negative effect of fertilization on richness was evident. These results indicate that at small scales species richness in mountain grasslands is determined by competition for light, which should be more important if nutrient availability is high, and by positive and negative interactions with unpalatable plants. In contrast, species richness at the large scale appears to be mainly influenced by land use. This result emphasizes the importance of studying such inter-relationships at multiple scales.

Our study further suggests that the maintenance of the traditional land use scheme is crucial for the conservation of plant species richness of mountain pastures as both intensification and abandonment changed species composition and reduced plant species diversity.

Introduction

In recent years changes in land use and other human activities have resulted in a decrease in species richness world-wide (Hooper et al. 2005 and references therein). Species richness is seen as an insurance against a decline in ecosystem services, such as the prevention of soil erosion and maintenance of hydrological cycles, or in ecosystem goods, such as tourism and recreation (Hooper et al. 2005). In Europe, the diversity of grasslands has dramatically decreased in lowland areas, whereas many traditionally managed mountain grasslands are still species-rich (MacDonald et al. 2000). However, changes in land use are threatening these habitats as well. At present we see increasing intensification of the use of well accessible sites paralleled by abandonment of less accessible sites in the European Alps (Tasser and Tappeiner 2002). When traditionally managed mountain grasslands below the tree line are abandoned, their floristic composition changes and their agricultural quality decreases, leading in the long term to reforestation and a significant reduction in biodiversity (Fischer and Wipf 2002). This development is difficult to reverse (Stampfli and Zeiter 1999), because of limited seed dispersal, recruitment and micro-site availability (Tilman 1997, Zobel et al. 2000). Intensification, in particular the application of fertiliser, has also been shown to decrease the species richness of mountain grasslands (Theodose and Bowman 1997, Nagy et al. 2003).

Most studies of the effects of land use change on plant species richness and vegetation composition in mountain grasslands were carried out at very small scales (Stampfli and Zeiter 1999, Fischer and Wipf 2002, Tasser and Tappeiner 2002, Dullinger et al. 2003, Müller et

al. 2003, Kleijn and Müller-Schärer in press). However, patterns in plant species richness and the underlying mechanisms can vary considerably among different spatial scales (Huston 1999 and references therein). At the scale of an individual pasture, the traditionally managed mountain grasslands are characterized by a large number of different microsites (Austrheim and Eriksson 2001, Erschbamer et al. 2003). This diversity of microsites can be explained by small-scale variation in topography and by the effects of grazing that increases the number of microhabitats through spatially heterogeneous defoliation, trampling, wallowing and faecal deposition (WallisDeVries et al. 1998). Heterogeneous sites consisting of a large number of different microsites are considered to support a large variety of plant species (Olff and Ritchie 1998). Both the fertilisation of grasslands, because it homogenises soil nutrients, and their abandonment, because the sites are no longer grazed, are likely to reduce the spatial heterogeneity. Hence, we hypothesize that species richness will decline in both cases. This process, however, may be scale-dependent since the degree of heterogeneity changes with spatial scales.

Unpalatable weeds are among the factors possibly influencing plant diversity and are strongly related to land use and land use change in mountain grassland. One of the most prominent representatives of unpalatable weeds in the European Alps is Veratrum album (Liliaceae), a large, highly toxic monocot native to Europe and Asia. It has attained pest status in France, Switzerland, Italy, Austria and Slovenia (FAO unpubl.). Because of its tall growth, it may negatively affect species richness at small scales simply due to competition for space (Crawley and Harral 2001). However, when

grazing is intense, unpalatable plants may act as facilitator species that enhance plant species richness (Bertness and Callaway 1994, Callaway et al. 2005). This suggests that the effect of unpalatable plants on species richness may depend on management. Furthermore, the interrelationship between species richness and weed abundance may also vary across spatial scales within a single management type. For example, while competition may lead to a negative relationship between weed abundance and species richness at small scales, extrinsic factors such as disturbance or propagule supply may affect species richness and weed abundance in a similar way at larger scales and thereby mask factors operating at neighbourhood scales (Levine et al. 2002). In a study in mountain grasslands of the Rocky Mountains, Stohlgren et al. (1999) found that native species richness and number of exotic weeds were negatively correlated at small scales (1 m²), but positively correlated at large scales (1000 m²).

The aim of this study was to investigate the effects of land use, soil properties, and the abundance of *V. album* on the species richness of mountain grasslands at four spatial scales. We addressed the following questions: 1) Is there an effect of land use on plant species richness and is this effect scale-dependent? 2) How well do management, soil chemistry and the abundance of *V. album* explain plant species richness at small and at large spatial scales? 3) Does the effect of *V. album* on plant species richness depend on land use and spatial scale?

Materials and methods

Study sites

Fifteen sites below the tree line with a high presence of the unpalatable plant *Veratrum album* (Liliaceae) were selected in five different regions of the Alps (Beaufortin, Bauges, Chablais in France, and Chablais and Lac de Dix in Switzerland, cf. Appendix 2-1). *Veratrum album* is an important weed of many mountain grasslands. It is typically found in natural grasslands above the tree line and in open woodlands, but has invaded semi-natural grasslands below the tree line. Large herbivores do not browse on *V. album* due to the high alkaloid concentration in all plant parts (Binns et al. 1972; for more details see Kleijn and Steinger 2002).

Within each region three sites were selected: a traditionally used unfertilised pasture (thereafter called ‘‘traditional pasture’’), a grazed and fertilised pasture (‘‘fertilised pasture’’), and a grassland that had been abandoned for at least 5 yr (‘‘abandoned pasture’’). In the following we refer to these three categories as ‘‘land use’’ and to grazing or fertilisation as ‘‘management’’. Fertilised grasslands had received either inorganic fertiliser or liquid manure for several years. Because abandoned pastures were rarest, they were chosen first. Then, the other two pasture types were selected within 5 km of this site, as similar as possible with regard to altitude, exposition and inclination. Information about time since abandonment, fertiliser application and duration of application were obtained from the farmers.

Vegetation and soil

At each site one modified Whittaker plot (MWP, Stohlgren et al. 1999) of 50 x 20 m was established in an homogeneously managed area (cf. Fig. 2-1). The minimum distance to adjacent areas with different land use was at least 15 m for abandoned sites and 50 m for sites with other management. The MWP was placed with its long side along the main slope. Nested in the MWP was one 100 m² subplot (5 x 20 m) in the centre and two 10 m² subplots (5 x 2 m) in opposite corners of the plot. Ten 1 m² subplots (each 0.5 x 2 m) were regularly spaced within the MWP, six of them along the inner border of the 1000 m2 plot and four along the outer border of the central 100 m² subplot. For each MWP, elevation and main exposition in degrees from north were recorded with a GPS and the inclination of the slope was calculated using the elevation of the upper and lower corner of the MWP recorded by the GPS. All sites were sampled in summer 2003 or 2004.

In the 1 m² subplots of the MWP, the foliar cover of all plant species and the area covered by bare ground and rocks were estimated to the nearest percent. Species with a cover of < 1% were assigned a cover value of 0.5%. Species richness (total number of species in a sample area) was recorded separately at each scale and each plot.

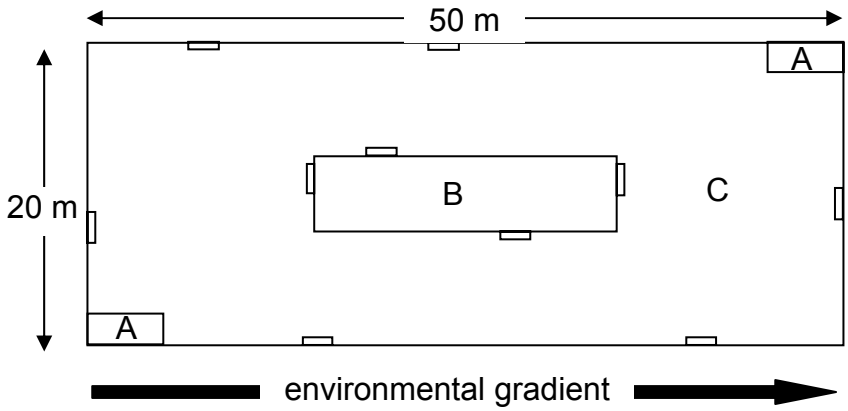


Fig. 2-1. Layout of the modified Whittaker plot (MWP) to study plant species diversity. The 1000 m² whole plot (C) contains one 100 m² subplot (B, 5 x 20 m), two 10 m² subplots (A, 5 x 2 m) and ten 1 m² subplots (0.5 x 2 m).

Twelve soil samples were taken in each 1 m² plot (ø 2.5 cm, depth 10 cm) to analyse soil chemical properties and moisture content. Soil samples were pooled per 1 m² plot, transferred to a deep-freezer (-18°C) within a few hours after sampling and kept frozen until further processing. NH_4 and NO_3-concentrations were measured with a colorimeter (Flow analyser, Skalar San Plus, The Netherlands) after solving 50 g soil in 250 ml of 1 m KCl, and ortho-phosphate with the Olsen method (Olsen et al. 1954). Soil pH was measured after shaking 1 ml soil in 5 ml distilled water (all analyses were carried out by SADEF, Aspach, France).

Statistical analysis

Cover data were used to calculate the Shannon-Wiener index of diversity ($H = - \sum p_i \ln p_i$, where p_i is the relative abundance of species i), and evenness ($H/H_{max} = H/\log S$, where S is the species richness). The mean Ellenberg light indicator value (mL, Ellenberg et al. 1991) was calculated for each 1 m² plot as $mL = \sum L_i \times p_i$, where L_i is the light indicator value of species i and pi its relative abundance. Mean Ellenberg nitrogen (mN), humidity (mH) and reaction indicator values (mR) were computed analogously. To elucidate differences among land use types with regard to site and soil characteristics, vegetation diversity indices, mean Ellenberg indicator values, and *V. album* and grass cover, ANOVAs were carried out using region (n = 5) and land use type (n = 3) as fixed factors. If an ANOVA revealed significant effects, Tukey's HSD test was used to identify significant differences between types of land use at the $p < 0.05$ level. The effect of management type (grazing, fertilisation) on species richness across multiple scales (1-1000 m²) was assessed using a hierarchical general linear model with region, grazing, fertilisation and log(area) as fixed

factors and site as a random factor. In this model, the sums of squares for each factor were adjusted for all factors that preceded it in the model.

To examine whether plant species richness was affected by different factors at different spatial scales, we calculated two separate hierarchical general linear models using the data from the smallest and the largest sampling scale (1 and 1000 m²). At the 1 m² scale, the model included region, grazing and fertilisation as fixed factors, site as a random factor, and nitrate, ammonium, total phosphate, pH, and cover of *V. album* as covariates. Soil variables were not or only moderately correlated with each other (all $r < 0.4$). We subsequently removed non-significant variables, but because we were specifically interested in the effect of *V. album* on plant species richness, we kept the cover of *V. album* and its interactions with grazing and fertilisation in the reduced models. Because the full model that included all soil variables at the 1 m² scale was not significantly different from the reduced model with nitrate as only soil variable (ANOVA, $p > 0.6$), the simpler model was preferred. Similarly, at the 1000 m² scale, the model contained region, grazing and fertilisation as fixed factors, and nitrate and cover of *V. album* as covariates.

To assess whether vegetation composition differed among land use types, the log-transformed cover values of the species in the 1 m² plots were analysed by the ordination technique non-metric multidimensional scaling (NMDS, Shepard 1962, Kruskal 1964), with the Bray-Curtis coefficient as distance measure. NMDS is commonly seen as the most robust unconstrained ordination method in community ecology (Minchin 1987). To find indicator species for the

different land use types, indicator species analyses (Dufrene and Legendre 1997) followed by a randomisation test were carried out with the log-transformed cover data. All statistical analyses were carried out using the R statistical language (Anon. 2004), except for the indicator species analyses which were carried out with PC-ORD (McCune and Mefford 1999).

Results

Site characteristics

Elevation, inclination of the slope, exposition of the MWPs, and all soil variables measured did not differ among the land use types (ANOVA, all $p > 0.15$), but elevation ($F_{4,8} = 7.4$) and exposition ($F_{4,8} = 7.1$, both $p = 0.01$) differed significantly among regions.

Plant species richness

Plant species richness was significantly lower in fertilised pastures than in traditional pastures pooled over all scales (Table 2-1, Fig. 2-2). The slope of the species-area relationship was lower in the fertilised than in the traditionally used, unfertilised pastures (see significant interaction between the effects of

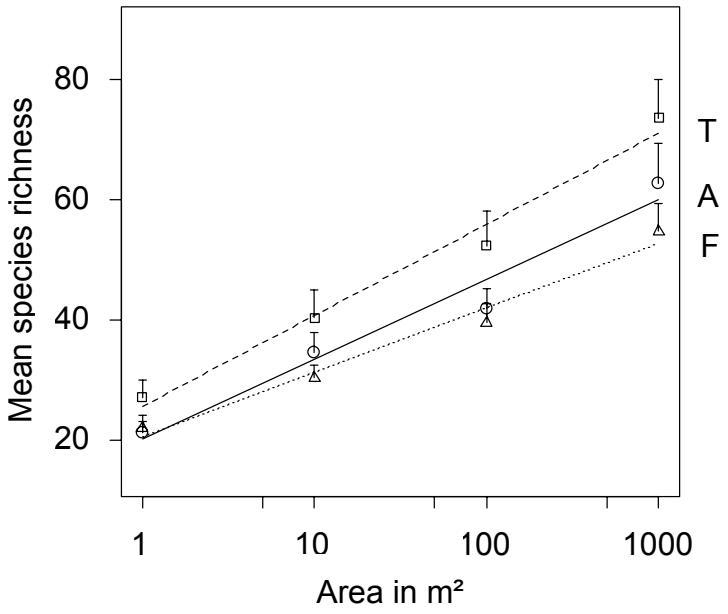


Fig. 2-2. Species – area relationship in mountain pastures of the Alps that had been subjected to different types of land use. Error bars indicate one standard error for each combination of land use type and area. Squares: traditional pastures (T); triangles: fertilised pastures (F); circles: abandoned pastures (A).

Table 2-1. General linear model of the effects of region, grazing, fertilisation and survey area on the number of plant species in mountain pastures of the Alps. Significant p-values (p <0.05) are in bold-face.

Source	df	F	p
Region	4	1.23	0.371
Grazing	1	0.32	0.586
Fertiliser (within Grazing)	1	5.63	**0.045**
Site	8	11.04	**<0.001**
Log (Area)	1	578.58	**<0.001**
Region x Log (Area)	4	7.80	**<0.001**
Grazing x Log (Area)	1	0.07	0.796
Fertiliser x Log (Area)	1	11.02	**0.002**
Residual	38		
Total	60		

fertilisation and spatial scale in Table 2-1, Fig. 2-2). As a consequence, species richness at 1000 m² was much higher in traditionally used grasslands (73.69±2.01 species) than in fertilised grasslands (54.89±1.48), while species richness at abandoned sites (62.89±2.10) was intermediate (ANOVA, $F_{2,8}$ = 4.26, p = 0.055; followed by Tukey's HSD test).

Plant species richness was influenced by different variables at different spatial scales (Table 2-2). At the 1 m² level, species richness

Table 2-2: General linear model of the effects of region, grazing, fertiliser, cover of *V. album*, and soil nitrate concentration on plant species richness in mountain pastures of the Alps at two spatial scales. Marginally significant p-values (0.1 >p >0.05) are in italics, significant p-values (p <0.05) are in bold-face.

	Species richness at 1 m²			Species richness at 1000 m²		
Source	df	F	p	df	F	p
Region	4	0.29	0.878	4	7.06	**0.042**
Grazing	1	1.08	0.330	1	0.14	0.725
Fertiliser	1	1.76	0.222	1	19.30	**0.012**
Site	8	23.09	**< 0.001**	---	---	---
Nitrate	1	9.81	**0.002**	1	4.65	*0.097*
Cover of *V. album*	1	4.51	**0.035**	1	1.20	0.336
V. album x Grazing	1	0.01	0.923	1	3.36	0.141
V. album x Fertiliser	1	2.89	*0.092*	1	5.06	*0.088*
Residual	130			4		
Total	149			15		

was negatively related to nitrate and cover of *V. album*. Nitrate concentration and cover of *V. album* were not correlated with each other ($p > 0.4$). At the 1000 m² level, species richness was negatively affected by fertilisation, and only marginally negatively correlated with nitrate.

Vegetation composition

Plant species composition did not differ between regions (ANOVA of NMDS scores along axis 1, $p > 0.05$), but was influenced by land use ($F_{2,8} = 4.61$, $p = .047$), probably due to change in grass cover. The cover of grasses was highest in fertilised pastures (49.99±1.3), lower in traditional pastures (40.89±1.4) and lowest in abandoned pastures (30.39±2.8) (ANOVA, $F_{2,8} = 4.91$, $p = 0.040$, followed by Tukey's HSD). Abandoned sites had significantly lower scores along the first NMDS axis than the two other types of land use (Tukey's HSD; Fig. 2-3). The scores along the first NMDS axis were negatively correlated with the Ellenberg indicator value for soil reaction ($r = 0.91$) and soil nitrogen ($r = 0.81$), suggesting that plants that typically grow at higher pH values and higher nutrient availability were more abundant in abandoned pastures. The mean light indicator value was positively related to the first NMDS axis ($r = 0.74$), suggesting that abandoned pastures contain more shade-tolerant plants than sites subjected to the two other types of land use (for all linear regressions: DF = 13, $p < 0.001$).

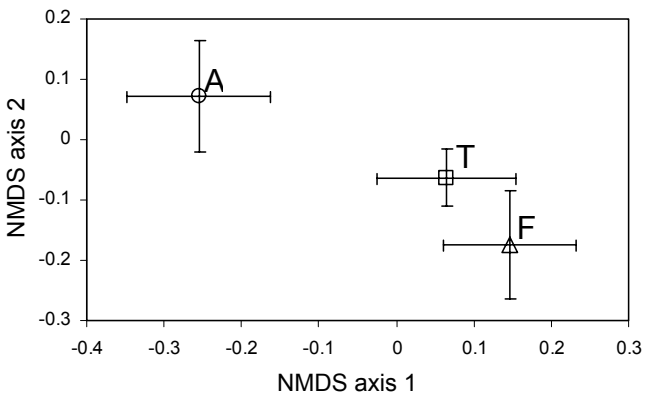


Fig. 2-3. Ordination diagram of a non-metric multi-dimensional scaling of the vegetational composition of mountain pastures in the Alps with different land use. Fertilised pastures (F); traditional pastures (T); abandoned pastures (A). The first NMDS axis was negatively correlated with the mean Ellenberg indicator value for soil reaction and soil nitrogen, and positively with the mean light indicator value.

Mean Ellenberg indicator values for soil reaction ($F_{2,8}$ = 4.39, p = 0.051) and light ($F_{2,8}$ = 3.34, p = 0.088) were marginally different between land use types. Abandoned pastures had the lowest indicator values for light (6.79±0.05) and the highest for soil reaction (6.49±0.05) and those were significantly different from those for the other two types of land use (fertilised pastures: mL 7.39±0.03, mR 5.59±0.15; traditional pastures: mL 7.19±0.06; mR 5.69±0.11).

Veratrum album and land use

The cover of *V. album* did not differ among land use types at any spatial scale (all p > 0.75), but the marginally significant interaction between the effects of fertilisation and cover of *V. album* at both scales on species richness (Table 2-2) suggested management-dependent effects of *V. album* on species richness. Linear regressions of species richness at the 1 m² scale on the abundance of *V. album* revealed different effects, depending on land use (Fig. 2-4). In abandoned pastures,

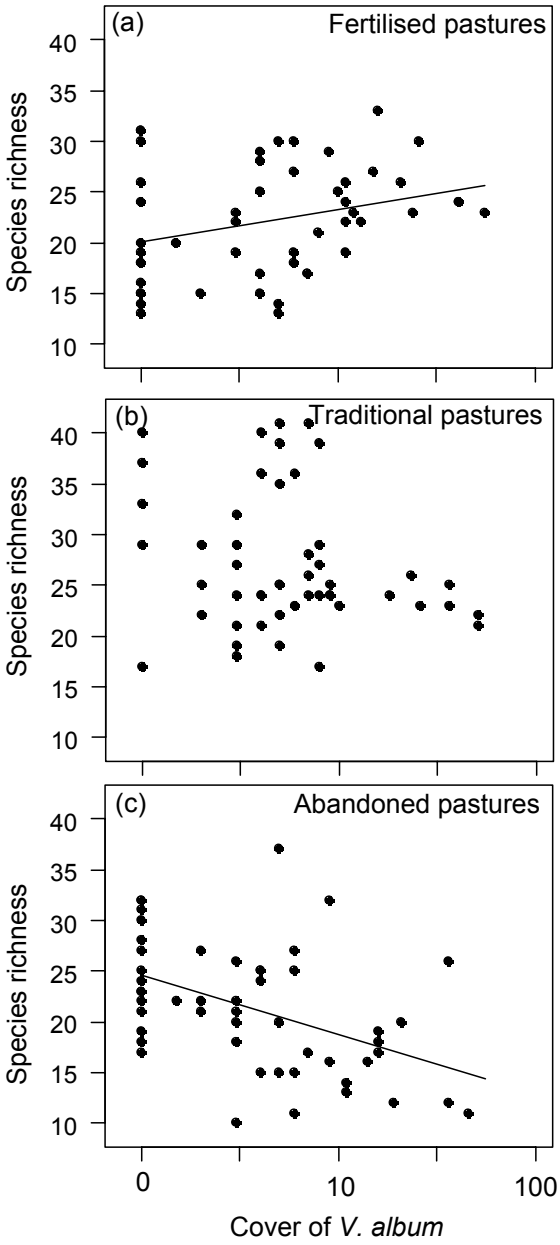


Fig. 2-4. The relationship between the species richness in 1 m² plots and the cover of the unpalatable plant *Veratrum album* for sites with different management in the Alps. (a) Fertilised (r = 0.30, p < 0.05), (b) traditionally used (p > 0.1), and (c) abandoned (r = -0.46, p < 0.001) mountain pastures (n = 50 for each type of land use).

species richness was negatively related to the cover of *V. album*, whereas the opposite was true for fertilised grasslands; in traditional pastures species richness was not related to the abundance of *V. album*. At the large scale (1000 m²) there was no relationship between species richness and *V. album* ($p > 0.3$), but statistical power was low ($n = 5$ for each land use type).

Discussion

Land use, spatial scale and vegetation

Our results suggest that the effect of land use on plant species richness in mountain grasslands is scale-dependent. Fertilised pastures had an overall lower species richness, and the increase in species richness with area was smaller in fertilised pastures than in traditional pastures (cf. Fig. 2-2, Table 2-1). The different increase in species richness with area may be explained by the varying degree of heterogeneity at a given spatial scale. At the smallest scale, the ecological interactions between individual plants appear to differ among land use types, as indicated by the different vegetation composition, but they result in a similar plant species richness. At the scale of pastures, factors such as geology, topography, hydrology and management are considered to be main determinants of plant species richness by creating a matrix of habitats with variable plant species composition (Crawley and Harral 2001). This is in agreement with our findings, indicating that at the scale of 1000 m² traditional pastures harbour a more diverse matrix of habitats than fertilized pastures. We hypothesize that fertiliser application has lead to the homogenisation of some of the heterogeneity initially present at the largest scale. As a

consequence, fertilised plant communities were dominated by a few plant species (mainly grasses) well adapted to the increased availability of nutrients and to intense grazing pressure, whereas the traditional pastures contained a high number of subdominant species with different micro-habitat requirements (Appendix 2-2).

Apart from spatial heterogeneity, species richness of grasslands may also be strongly influenced by the disturbance regime (Milchunas et al. 1988). In agreement with the intermediate disturbance hypothesis (Connell 1978, Huston 1979), we found the species richest communities in traditional pastures, where the level of disturbance is intermediate between that at fertilized and abandoned sites. Grazing pressure and thus disturbance is high in fertilised pastures, whereas at abandoned sites there is no grazing and thus little disturbance. In fertilized pastures mainly grasses were found as characteristic species (Appendix 2-2). In mountain grasslands, most forbs are adapted to low nutrient conditions and less tolerant to grazing than grasses (Oksanen 1990, Oksanen and Moen 1994). At fertilised sites with intense grazing pressure grasses may thus outcompete the less grazing-tolerant forbs. In the case of abandoned sites, shade-tolerant species (e.g. *Chaerophyllum hirsutum*, *Geranium sylvaticum*, *Equisetum sylvaticum*; Appendix 2-2) were more dominant, suggesting increased competition for light.

The plant community of abandoned grasslands differed from that of traditional and fertilised pastures, although the sites had only been abandoned for 5-40 yr. In mountain grasslands of the Alps shifts in vegetational composition can occur rapidly and may be already detectable four years after abandonment (Stampfli 1992). The

absence of continuous grazing by livestock allowed some common forest species to establish and spread, but at the same time reduced the abundance of a number of uncommon or rare plant species characteristic for traditional pastures, such as *Arnica montana* and *Orchis maculata* (Appendix 2-2). Thus, abandonment resulted in a decrease in conservation value due to changes in species composition even though species richness did not decline significantly.

Scale-dependent response of species richness

Our study indicates that factors influencing species richness in mountain grasslands vary with scale, probably due to different mechanisms operating at different scales. For example, at small spatial scales interactions between *V. album* and other plant species as well as soil nitrate concentration influenced species richness. At small spatial scales in grasslands, competition for space generally is very important (Tilman 1994). *Veratrum album* is one of the tallest and largest plants of its community. It is also one of the first plants that start growing in spring and it reaches its maximal shoot biomass about two months before most other plants (Kleijn and Müller-Schärer in press). It therefore may have a competitive advantage over smaller forbs and grasses due to asymmetric competition for light (Newman 1973).

Ammonium was the predominant form of inorganic soil nitrogen in our study sites, which is typical for many mountain areas (Körner 1999). Nevertheless, species richness was not influenced by ammonium, but by nitrate levels. Nutrient enrichment experiments at arctic and alpine sites revealed that a higher availability of nitrogen increases the productivity of these sites (Nordin et al. 2004, van Wijk

et al. 2004) and alters their species composition (Graglia et al. 2001, Richardson et al. 2002). McKane et al. (2002) provide evidence that plants growing in cold climatic zones differ in timing, chemical form, and depth of nitrogen uptake. It remains unclear, though, why particular forms of nitrogen should be more strongly linked to species richness than others. Probably, high nitrate availability mainly increases grass biomass, which may lead to an out-shading of less competitive forb species (Willems et al. 1993).

At the 1000 m² scale, the only significant factor explaining species richness was fertiliser application. It should be noted, though, that the statistical power to detect effects of the covariates at the 1000 m² scale was much lower than at the 1 m² scale (denominator DF at 1000 m² = 4; at 1 m² = 130). This may have influenced the results and explain why nitrate and the *V. album* x fertiliser interaction were only marginally significant at the 1000 m² scale. Nevertheless, our results indicate that management interventions such as fertiliser application, which usually are carried out at the level of whole pastures, are more important determinants of large-scale species richness than parameters describing small-scale resource availability.

The interacting effects of *Veratrum album* and land use on species richness

Our study provides evidence that, apart from the well established direct effects of land use on species richness, there is also a link between land use and species richness via ‘‘mediator species’’ which promote or reduce species richness depending on land use. In our study, species richness was positively related to the abundance of the toxic *V. album* in fertilised pastures, where grazing pressure was

strong. Our findings are in agreement with the results of Callaway et al. (2000) and Smit et al. (2006) and suggest indirect facilitation of other species by *V. album*. However, at abandoned sites species richness decreased with increasing abundance of *V. album* suggesting competition. At such sites *V. album* may out-compete other species due to decreased light availability under *V. album* (Kleijn and Müller-Schärer in press). Similarly, Callaway et al. (2005) reported that removing *Veratrum lobelianum* from sites where cattle grazing was permitted reduced species richness, whereas inside an exclosure the removal of *Veratrum* increased community richness. *Veratrum album* is generally seen as an undesirable species for agriculture, as it reduces fodder quality, but like other unpalatable species (Ellenberg 1989, Callaway et al. 2000), it may have positive effects on plant diversity if grazing is intense (Bertness and Callaway 1994).

At large scales, we found neither a relationship between species richness and the abundance of *V. album* nor between the land use types and the abundance of *V. album*, but both might be due to the low statistical power. One could assume that pastures with a high abundance of weeds are preferentially abandoned because of their low forage quality. We did not find support for this in our study, since the cover of *V. album* was similar at sites with different land use. Nevertheless, it is likely that, once a pasture has become invaded by *V. album*, cattle stocking will be reduced due to decreased forage quality. This in turn may promote the further spread of *V. album*, because trampling by cattle limits establishment of seedlings of *V. album* (Treier pers. comm.). Moreover, the presence of *V. album* increases the survival of tree saplings (Smit et al. 2006) in grazed

pastures, and may therefore accelerate the process of a gradual abandonment and of reforestation of mountain grasslands.

Conclusions

Our study across five regions of the Alps indicates that different mechanisms influence species richness at different spatial scales. Unpalatable plants play an important role as mediator species by modifying the effect of land use on plant species diversity. At small scales nutrient availability and positive and negative interactions with unpalatable species determine species richness. *Veratrum album* reduces species richness in abandoned pastures, but preserves plant diversity when grazing is intense. At larger scales, plant species richness and vegetational composition are determined by management. This finding emphasizes the importance of studying effects of land use on species diversity especially at larger scales. Maintaining the traditional land use appears to be the most promising approach to conserve the high biodiversity of mountain grasslands.

Acknowledgements

This work was funded by the Swiss Priority Program (NFP 48) ‘‘Landscapes and Habitats of the Alps’’, a research project of the Swiss National Science Foundation (grant 4048-064424 to HMS and US). Field work was also supported by a travel grant from the Swiss Academy of Science to TS.

References

Austrheim, G. and Eriksson, O. 2001. Plant species diversity and grazing in the Scandinavian mountains - patterns and processes at different spatial scales. - Ecography 24: 683-695.

Bertness, M. D. and Callaway, R. M. 1994. Positive interactions in communities. - Trends Ecol. Evol. 9: 191-193.

Binns, W., Keeler, R. F. and Balls, L. D. 1972. Congenital deformation in lambs, calves and goats resulting from maternal ingestion of *Veratrum californicum*: hare clip, cleft palate, ataxia and hypoplasis of metacarpal and metatarsal bones. - Clinical Toxicol 5: 245-261.

Callaway, R. M., Kikodze, D., Chiboshvili, M. and Khetsuriani, L. 2005. Unpalatable plants protect neighbors from grazing and increase plant community diversity. - Ecology 86: 1856-1862.

Callaway, R. M., Kikvidze, Z. and Kikodze, D. 2000. Facilitation by unpalatable weeds may conserve plant diversity in overgrazed meadows in the Caucasus Mountains. - Oikos 89: 275-282.

Crawley, M. J. and Harral, J. E. 2001. Scale dependence in plant biodiversity. - Science 291: 864-868.

Dufrene, M. and Legendre, P. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. - Ecol. Monogr. 67: 345-366.

Dullinger, S., Dirnböck, T., Greimler, J. and Grabherr, G. 2003. A resampling approach for evaluating effects of pasture abandonment on subalpine plant species diversity. - J. Veg. Sci. 14: 243-252.

Ellenberg, H. 1989. *Opuntia dillenii* as a problematic neophyte in the Yemen-Arab-Republic. - Flora 182: 3-12.

Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W. and Paulißen, D. 1991. Zeigerwerte von Pflanzen in Mitteleuropa. - Goltze.

Erschbamer, B., Virtanen, R. and Nagy, L. 2003. The impacts of vertebrate grazers on vegetation in Europe high mountains. - In: Nagy, L., Grabherr, G., Körner, C. and Thompson, D. B. A. (eds.), Alpine Biodiversity in Europe. Springer, pp. 377-396.

Fischer, M. and Wipf, S. 2002. Effect of low-intensity grazing on the species-rich vegetation of traditionally mown subalpine meadow. - Biol. Conserv. 104: 1-11.

Graglia, E., Jonasson, S., Michelsen, A., Schmidt, I. K., Havstrom, M. and Gustavsson, L. 2001. Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. - Ecography 24: 5-12.

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J. and Wardle, D. A. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. - Ecol. Monogr. 75: 3-35.

Huston, M. A. 1979. A general hypothesis of species diversity. – Am. Nat. 113: 81-101.

Huston, M. A. 1999. Local processes and regional patterns: appropriate scales for understanding variation in the diversity of plants and animals. - Oikos 86: 393-401.

Kleijn, D. and Müller-Schärer, H. 2006. The relation between unpalatable species, nutrients and plant species richness in Swiss montane pastures. - Biodivers. Conserv. in press.

Kleijn, D. and Steinger, T. 2002. Contrasting effects of grazing and hay cutting on the spatial and genetic populations structure of *Veratrum*

album, an unpalatable, long-lived, clonal plant species. - J. Ecol. 90: 360-370.

Körner, C. 1999. Alpine plant life. - Springer Verlag.

Kruskal, J. B. 1964. Non-metric multidimensional scaling: a numerical method. - Psychometrika 29: 115-129.

Levine, J. M., T. Kennedy, and S. Naeem. 2002. Neighbourhood scale effects of species diversity on biological invasions and their relationship to community patterns in M. Loreau, et al. (eds), Biodiversity and ecosystem functioning - synthesis and perspectives. Oxford Univ. Press, pp. 11-124.

MacDonald, D., Crabtree, J. R., Wiesinger, G., Dax, T., Stamou, N., Fleury, P., Gutierrez Lazapita, J. and Gibon, A. 2000. Agriculture abandonment in mountain areas of Europe: Environmental consequences and policy response. - J. Environ. Manage. 59: 47-69.

McCune, B. and Mefford, M. J. 1999. PC-ORD for Windows.

McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., Giblin, A. E., Kielland, K., Kwiatkowski, B. L., Laundre, J. A. and Murray, G. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. - Nature 415: 68-71.

Milchunas, D. G., O. E. Sala, and W. K. Lauenroth. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. Am. Nat. 132: 87-106.

Minchin, P. R. 1987. An evaluation of relative robustness of techniques for ecological ordinations. - Vegetatio 7: 145-156.

Müller, P., Güsewell, S. and Edwards, P. J. 2003. Einfluss von Boden und Bewirtschaftung auf die Artenvielfalt der Vegetation auf Alpweiden im Glarnerland. - Botanica Helvetica: pp. 15-36.

Nagy, L., G. Grabherr, C. Körner, and D. B. A. Thompson (eds). 2003. Alpine biodiversity in Europe. - Springer.

Newman, E. I. 1973. Competition and diversity in herbaceous vegetation. - Nature 244: 310-311.

Nordin, A., Schmidt, I. K. and Shaver, G. R. 2004. Nitrogen uptake by artic soil microbes and plants in relation to soil nitrogen supply. - Ecology 85: 955-962.

Oksanen, J. 1990. Predation, herbivory, and plant strategies along gradients of primary production. – In: Grace, J. and Tilman, D. (eds), Perspectives on plant competition. Academic Press, pp 445-474.

Oksanen, L., and J. Moen. 1994. Species-specific plant responses to exclusion of grazers in three Fennoscandian tundra habitats. - Ecoscience 1: 31-39.

Olff, H. and Ritchie, M. E. 1998. Effects of herbivores on grassland plant diversity. - Trends Ecol. Evol. 13: 261-265.

Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean, L. A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. - U.S. Dep. of Agric. Circ. 939.

Richardson, S. J., Press, M. C., Parsons, A. N. and Hartley, S. E. 2002. How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. - J. Ecol. 90: 544-556.

Shepard, R. N. 1962. The analysis of proximities: multidimensional scaling with an unknown distance function. - Psychometrika 27: 125-139.

Smit, C., den Ouden, J. and Müller-Schärer, H. 2005. Unpalatable plants facilitate tree sapling survival in wooded pastures. - J. Appl. Ecol., in press.

Stampfli, A. 1992. Year-to-year changes in unfertilized meadows of great species richness detected by point quadrate analysis. - Vegetatio 103: 125-132.

Stampfli, A. and Zeiter, M. 1999. Plant species decline due to abandonment of meadows cannot easily be reversed by mowing. A case study from the southern Alps. - J. Veg. Sci. 10: 151-164.

Stohlgren, T. J., Binkley, D., Chong, G. W., Kalkhan, M. A., Schell, L. D., Bull, K. A., Otsuki, Y., Newman, G., Bashkin, M. and YowHan, S. 1999. Exotic plant species invade hot spots of native plant diversity. - Ecol. Monogr. 69: 25-46.

Tasser, E. and Tappeiner, U. 2002. Impact of land use changes on mountain vegetation. - Appl. Veg. Sci. 5: 173-184.

The R Development Core Team. 2004. R: A language and environment for statistical computing. - R Foundation for Statistical Computing.

Theodose, T. A., and Bowman, W. D. 1997. Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. Ecology 78:1861-1872.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. - Ecology 75: 2-16.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. - Ecology 78: 81-92.

van Wijk, M. T., E. Clemmensen, G. R. Shaver, M. Williams, T. V. Callaghan, F. S. Chapin, J. H. C. Cornelissen, L. Gough, S. E. Hobbie, S. Jonasson, J. A. Lee, A. Michelsen, M. C. Press, S. J. Richardson, and H. Rueth.2004. Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: generalizations and differences in ecosystem and plant

type responses to global change. - Global Change Biol. 10: 105-123.

WallisDeVries, M. F., Bakker, J. P. and Van Wieren, S. F. (eds.). 1998. Grazing and Conservation Management. - Kluwer Academic Publishers.

Willems, J. H., Peet, R. K. and Bik, L. 1993. Changes in chalk-grassland structure and species richness resulting from selective nutrient additions. - J. Veg. Sci. 4: 203-212.

Zobel, M., Otsus, M., Liira, J., Moora, M. and Möls, T. 2000. Is small-scale species richness limited by seed availability or microsite availability? - Ecology 81: 3274-3282.

Appendix 2-1. Description of study sites. Country (CH: Switzerland; F: France), region, land use type, mean elevation, inclination, exposition, cover of *V. album*. Type of land use: t, traditional; f, fertilised; a, abandoned pasture.

Country	Region	Landuse	Elevation	Inclination	Exposition	% Cover *V. album*
F	Bauges	a	1755 m	36%	NW	3.2
F	Bauges	t	1607 m	27%	W	3.5
F	Bauges	f	1628 m	18%	W	15.9
F	Beaufortin	a	1838 m	29%	WNW	3.6
F	Beaufortin	t	1891 m	23%	NNW	3.5
F	Beaufortin	f	1914 m	25%	NWN	3.3
F	Chablais	a	1470 m	36%	NNW	2.0
F	Chablais	t	1470 m	36%	NNW	4.0
F	Chablais	f	1550 m	36%	NNW	3.3
CH	Chablais	a	1517 m	31%	WSW	24.3
CH	Chablais	t	1409 m	20%	W	24.6
CH	Chablais	f	1539 m	47%	W	4.3
CH	Lac de Dix	a	1507 m	79%	SW	4.5
CH	Lac de Dix	t	1646 m	32%	WSW	5.5
CH	Lac de Dix	f	1255 m	36%	NW	4.3

Appendix 2-2. Indicator species analysis (Dufrene and Legendre 1997) for plant species of mountain grasslands in the Alps. All p-values < 0.01. Species are arranged according to the land use type they indicate (t, traditional pastures; f, fertilised pastures; a, abandoned pastures).

Species	Indicator species for	Cumulative presence			Mean Cover		
		f	t	a	f	t	a
Festuca rubra	f	46	45	35	9.4	8.6	3.1
Cynosurus cristatus	f	35	21	19	6.0	2.6	2.2
Anthoxanthum odoratum	f	29	21	12	3.6	1.7	1.2
Taraxacum officinale	f	25	10	10	1.4	0.5	0.6
Luzula alpinopilosa	f	24	22	1	2.0	0.9	0.1
Leontodon autumnalis	f	22	8	1	1.1	0.3	0.1
Carum carvi	f	17	7	3	2.7	0.4	0.0
Phleum phleoides	f	10	0	0	3.1	0.0	0.0
Knautia dipsacifolia	f	7	0	1	0.1	0.0	0.0
Trifolium pratensis	t	36	38	15	1.7	2.6	0.8
Leucanthemum vulgare	t	5	21	12	0.1	0.5	0.3
Lotus corniculatus	t	8	18	4	0.3	0.9	0.1
Geum montanum	t	5	17	3	0.2	0.5	0.1
Crepis aurea	t	6	16	0	0.1	0.7	0.0
Homogyne alpina	t	1	15	4	0.0	0.6	0.2
Phyteuma orbiculare	t	4	14	2	0.1	0.4	0.0
Cirsium acaule	t	0	14	0	0.0	0.7	0.0
Hieracium lactucella	t	6	12	0	0.2	0.6	0.0
Polygala vulgaris	t	0	10	1	0.0	0.2	0.0
Phyteuma betonicifolium	t	0	10	0	0.0	0.2	0.0
Arnica montana	t	0	10	0	0.0	1.5	0.0
Alchemilla flabellata	t	0	10	0	0.0	0.5	0.0
Poa pratensis	t	0	9	2	0.0	2.2	0.4
Myosotis sylvatica	t	1	9	0	0.0	0.2	0.0
Galium mullogo	t	0	9	1	0.0	0.1	0.0
Holcus mollis	t	0	8	1	0.0	1.2	0.0
Centaurea pseudophrygia	t	3	8	0	0.2	0.8	0.0
Carex sempervirens	t	0	8	0	0.0	0.5	0.0
Orchis mascula	t	0	6	0	0.0	0.1	0.0
Gagea fistulosa	t	0	6	0	0.0	0.1	0.0
Deschampsia caespitosa	a	7	25	28	0.7	4.2	8.4
Chaerophyllum hirsutum	a	33	43	48	1.9	4.6	6.5
Geranium sylvaticum	a	22	34	39	1.4	2.5	5.1
Heracleum sphondylium	a	2	0	7	0.1	0.0	2.6

Species	Indicator species for	Cumulative presence			Mean Cover		
		f	t	a	f	t	a
Phleum pratensis	a	0	10	16	0.0	0.9	1.6
Rubus sp.	a	0	0	7	0.0	0.0	1.3
Bromus erectus	a	3	0	8	0.1	0.0	1.2
Equisetum sylvaticum	a	0	0	8	0.0	0.0	0.8
Hypericum maculatum	a	9	14	21	0.1	0.2	0.7
Carex montana	a	0	0	6	0.0	0.0	0.6
Rosa canina	a	0	0	8	0.0	0.0	0.4
Myosotis decumbens	a	0	0	8	0.0	0.0	0.4
Centaurea jacea	a	6	2	15	0.1	0.0	0.4
Cardamine pratensis	a	0	0	8	0.0	0.0	0.1

Long-term effects of short-term perturbation in a sub-alpine grassland

Thomas Spiegelberger, Otto Hegg, Diethart Matthies, Katarina Hedlund and Urs Schaffner

Paper published in *Ecology*, **87**, pp. 1939-1944 (2006)

Abstract

Theoretical advances and short-term experimental studies have furthered our understanding of how ecosystems respond to perturbation. However, there are few well-replicated experimental studies that allow an assessment of long-term responses. Results from a controlled, large-scale field experiment in a subalpine grassland near Interlaken, Switzerland, show that 2–4 years of liming (Ca: 40 g/m² yr^1) still significantly affected the composition of the vegetation and the soil microbial community nearly 70 years after the treatments were imposed, whereas NPK fertilization (8 g/m² yr^1) only marginally affected vegetation composition. The exchangeable content of Ca ions and soil pH were higher in limed plots, but were unaffected in fertilized plots. Plant species and PLFAs (phospholipid fatty acids) indicating low pH values were found in higher abundance in the unlimed plots, suggesting that the long-lasting effects of liming on the above- and belowground communities were mediated through changes in soil pH. The results of this long-term study indicate that the resilience of mountain ecosystems may be particularly low in response to perturbations that substantially alter soil pH or other key determinants of belowground processes.

Introduction

Long-term experiments in seminatural and natural terrestrial ecosystems have revealed that continuous human influences, such as the input of nutrients or acid rain, can have dramatic effects on the composition of communities and on ecosystem functioning (Tilman and Lehman 2001). For example, the Park Grass experiments at Rothamsted, UK, which started in 1856, have revealed that continuous fertilizer or liming amendments to the soil significantly changed soil pH, the composition of the vegetation and the soil microbial community, plant species richness, litter decomposition, and soil development (Johnston et al. 1986, Tilman et al. 1994). However, little is known about whether ecosystems are resilient in the long-term against short-term perturbations (Scheffer et al. 2001). Two types of resilience have been proposed. After disturbance components of resilient ecosystems may either return to their original state (resilience sensu May 1973), or may undergo a transition toward an alternative stability domain (ecological resilience sensu Holling 1973; see also Gunderson 2000 and Scheffer et al. 2001).

Theoretical and experimental work suggest that resilience is affected by a number of ecosystem characteristics, including nutrient cycling (DeAngelis et al. 1989, Moore et al. 1993), plant functional types (Leps et al. 1982, MacGillivray and Grime 1995), and ecological diversity (Tilman and Downing 1994). The ‘‘resilience – productivity hypothesis’’ posits that the time required by an ecosystem to regain equilibrium after a disturbance is similar to the turnover time of nutrients in the system (DeAngelis et al. 1989, Moore et al. 1993), suggesting that resilience may be particularly low in ecosystems with

harsh environmental conditions, like alpine and arctic ecosystems. Plant species growing in alpine and arctic ecosystems are characterized by a set of traits that promote the tolerance of environmental stresses (Körner 1999). The same traits are associated with low growth rates and are therefore predicted to be associated with low rates of resilience (Leps et al. 1982, MacGillivray and Grime 1995). Moreover, resilience is likely to vary not only among ecosystems, but also among components and processes within the same ecosystem (Lavorel et al. 1999). For example, recovery of vegetation cover may be relatively fast, while recovery of vegetational composition might be considerably slower.

Here, we report results from a controlled, large-scale field experiment on the long-term effects of liming and fertilization on the composition of the plant and microbial community of a subalpine grassland. The treatments were carried out from 1932 to 1935 and led to a significant shift in plant species composition within a few years. We revisited the experimental site nearly 70 years after the treatments were imposed to test the hypothesis that liming and NPK fertilization still affected vegetation composition. We also aimed to test whether the short-term addition of lime and NPK fertilizer had long-term effects on soil chemical properties and on the composition of the soil microbial community.

Materials and methods

The experiment at the Schynige Platte near Interlaken (Bernese Oberland, Switzerland) was set up in 1930 by Werner Lüdi at 1925 m above sea level in a subalpine acid grassland (acid cambisol, pH at the beginning of the experiment 4.5–5.0 [Lüdi 1948]) on a south-southeast-facing slope with an inclination of 20°. Mean annual precipitation is ~1800 mm, and mean annual temperature is ~18C. Before 1930, the site had been used as a pasture for many centuries. Once the experimental plots had been set up and the treatments initiated, the whole site was fenced, and was mowed once a year in late summer. From 1958 to 1980 the site was grazed by cattle. Since 1980, the site has been fenced again and maintained by one of the authors (O. Hegg) by mowing it once a year. The vegetation of the site consisted mainly of grasses, in particular *Nardus stricta* L. and *Festuca rubra* L. with an average cover of ~30% and 5%, respectively. Subordinate species with an average cover of between 3% and 5% were *Arnica montana* L., *Crepis conyzifolia* (Gouan) Kerner, *Gentiana purpurea* L., and *Vaccinium myrtillus* L. Bryophytes and lichens were rare in the experimental plots and were not recorded.

We present results from a subset of 80 plots out of the 340 plots set up by W. Lüdi. The plots, each 2.56 m² (1.6 x 1.6 m), separated by 0.4 m wide access paths, were arranged in four blocks. Each block consisted of 20 plots, which were subjected in a factorial design to the two treatments: liming (yes/no) and NPK fertilizer (yes/no). Both treatments were applied in all blocks once a year in early summer 1932 and 1933. In two of the four blocks, the treatments were repeated in 1934 and 1935. N was applied as ammonium sulphate, P as

superphosphate, and K as potassium (Hegg et al. 1992). The fertilized plots also received a small amount of Ca as part of the P fertilizer. In total, plots that were limed over a period of two years received 80 g/m² Ca, and plots that were fertilized received 1.4 g/m² N, 4.9 g/m² P, 9.7 g/m² K, and 19 g/m² Ca. Plots that were treated over a period of four years received twice the amount of nutrients compared to those treated over a period of two years. The 20 plots of each block were arranged in two parallel rows of 10 plots along the main altitudinal gradient. The four treatment combinations were allocated to the plots in a regular design.

In summer 2002 complete plant species lists were established for the central 1-m² square of each plot. Then, the cover of each vascular plant species was estimated to the nearest 1% (Mueller-Dombois and Ellenberg 1974). Cover for species with ~1% cover was assessed with greater precision by placing a 10 x 10 cm square frame (1% of the 1-m² square) over each plant of a species and summing the estimated cover for all plants of that species. Cover was evaluated for each species independently so that the sum of cover values could exceed 100% and reflect canopy layering. Soil samples were collected on 9 August 2003. In each plot, four soil cores (diameter 18 mm, depth 10 cm) were taken with a metal sampler next to the four corners of the central 1-m² subplot, pooled, and transferred to a deep-freezer (-18°C) within a few hours after sampling. The soil samples were then homogenized and sieved (mesh size 2 mm). Organic matter content was determined by reweighing 3 g of dry soil after burning at 400°C for 8 h. The exchangeable content of Ca and K was determined in an inductively coupled plasma spectrophotometer (Optima 3000 DV, PerkinElmer, Wellesley, Massachusetts, USA) after dissolving 5 g of dry soil for 2 h in 0.1 mol/L $BaCl_2$. To make the soil pH measurements

comparable with those from previous studies (Lüdi 1959, Hegg et al. 1992), pH was measured after shaking 5 g of soil in 5 mL of distilled water for 15 min. Lipid extractions were made from 3 g soil as described by Hedlund (2002). The sum of the phospholipid fatty acids (PLFA) i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0 was used as an index of bacterial biomass (Frostegård and Bååth 1996). The amount of PLFA 18:2ω6 was used as an index of saprophytic fungal biomass and the neutral lipid fatty acid (NLFA) 16:1ω5 as a marker for arbuscular mycorrhizal fungi (Olsson 1999, Hedlund 2002). All PLFA and NLFA concentrations were expressed as nanomoles per gram of dry soil.

To assess the productivity of the 80 plots, a bioassay was carried out using *Festuca rubra* L. as a phytometer. A bioassay was preferred to an analysis of soil nitrogen, because nutrient availability for plants is often only weakly related to element concentrations in the soil (Schimel and Bennett 2004). *F. rubra* is a species with a wide ecological amplitude with respect to pH (Ellenberg et al. 1991). Sieved and homogenized soil samples from each of the 80 plots were placed in plastic tubes (diameter 2 cm, height 8 cm), which were tightly closed by a 55-µm gauze screen at the bottom. The tubes were individually placed in cups and randomly arranged on a greenhouse bench. Two seedlings of *F. rubra* were planted in each tube. Seedlings that died within five days after transplanting were replaced. Plants received regularly the same amount of water. After 10 weeks the seedlings were harvested, dried for 48 hours at 80°C, and weighed.

We subdivided all experimental blocks into an upper and a lower half to take into account the altitudinal gradient at the site, which

resulted in a total of eight blocks containing 2–3 replicates of each of the four treatment combinations. All analyses were based on 80 soil samples and 79 vegetation records (one census was lost). The statistical power to detect the effects of factors that varied at the block level (duration of fertilizer application, i.e., two or four years, and position of plot along the slope) was very low due to low replication, and no effects were found. All analyses were therefore carried out using the factors block, fertilization, and liming. Univariate data were analyzed by analyses of variance using the R-1.8.1 statistical language (R Development Core Team 2004). To fulfill the assumptions of normally distributed residuals and homogeneity of variances data were transformed in some cases prior to analysis.

For the multivariate analyses of community composition, the data on individual plant cover were log-transformed, and the individual PLFA amounts were divided by the sum of all PLFAs and then also log-transformed prior to analysis. Initial detrended correspondence analyses showed that beta diversity (gradient length) of the communities was relatively low (1.83), and following the recommendations of Leps and Smilauer (2003) the data were therefore analyzed by redundancy analysis, a constrained form of principal component analysis (van den Wollenberg 1977). The method used by us to record the vegetation (Mueller-Dombois and Ellenberg 1974) includes a visual and therefore potentially subjective estimation of the cover of the individual plant species, but has the advantage of providing a complete species list for each plot. To test for a potential bias due to the visual estimation of species cover, we also carried out a canonical correspondence analysis of the vegetation composition using species presence–absence data. All multivariate analyses were

carried out using CANOCO 4.02 (ter Braak and Smilauer 1999). The significance of the treatments was tested using the randomization test available in CANOCO.

To identify plant species and phospholipid fatty acids indicating the treatments imposed, indicator species analyses (Dufrene and Legendre 1997) followed by randomization tests were carried out with the log-transformed data using PC-ORD (McCune and Mefford 1999). Indicator species analysis combines information on the relative abundance of a species and its relative frequency of occurrence in groups of sites (Dufrene and Legendre 1997).

Results

Nearly 70 years after the last liming treatment, both the soil pH (5.14±6 0.04 vs. 4.97±0.04, $F_{1,69}$ = 13.0, P < 0.001) and the exchangeable content of Ca ions (181±9.0 vs. 148±9.6 mg/L, $F_{1,69}$ = 9.9, P = 0.002) were significantly higher in limed than in unlimed plots (data are given as means and standard errors). In contrast, fertilization had no effect on either the pH or the exchangeable content of Ca ions (both P > 0.5). Furthermore, K ions and organic matter content were not significantly affected by any of the treatments (all P > 0.1), and treatments did not influence the aboveground biomass of the phytometer *F. rubra* in the bioassay (all P > 0.15). Plant species richness per plot increased with soil pH (linear regression, r = 0.27, P = 0.009).

Liming still had a highly significant effect on vegetation composition 70 years after the last treatment (Table 3-1: Plant community). In contrast, the effect of fertilization was only significant at the 10% level. Similar results were obtained when species presence–absence data were used instead of cover values (canonical correspondence analysis: liming, $F_{1,68}$ = 2.12, P = 0.002; fertilization, $F_{1,68}$ = 1.23, P = 0.114), indicating that limed and unlimed plots differed in terms of the presence of plant species. Indicator species analysis identified 13 species that were significantly more abundant either in limed plots or in unlimed plots (Table 3-2). The indicator species for liming are known as indicators for neutral soil reaction from the literature, whereas those indicating no liming are typical species of acid soils (Table 3-2), suggesting that the change in vegetation composition was due to the increased pH. However, when soil pH was included in the redundancy analysis as a covariate, the effect of liming was still significant ($F_{1,67}$ = 2.0, P = 0.003). Plant species richness tended to be higher in limed plots than in unlimed plots (31.6±0.61 species vs. 29.8±0.73 species, $F_{1,68}$ = 3.1, P = 0.08), but was not affected by fertilization (P = 0.97).

Table 3-1. Results of redundancy analyses of the effects of NPK-fertilization and liming in the 1930s on the composition of the plant community of a subalpine pasture in 2002, and on the microbial community in 2003. *P* values are derived from randomization tests (ter Braak and Smilauer 1999). Significant test results are in boldface type.

	Plant community (2002)				Soil microbial community (2003)			
Source	df	SS	*F*	*P*	df	SS	*F*	*P*
Block	7	0.181			7	0.174		
NPK	1	0.015	1.4	0.083	1	0.013	1.1	0.331
Liming	1	0.042	3.8	**<0.001**	1	0.029	2.6	**0.009**
NPK x Lim.	1	0.008	0.7	0.898	1	0.005	0.4	0.920
Error	68	0.754			69	0.779		
Total	78	1.000			79	1.000		

Table 3-2. Indicator plant parameters for liming and no-liming treatments revealed by indicator species analysis (Dufrene and Legendre 1997). The indicator value is a measure combining information on the relative frequency and relative abundance of a species in a group of sites. Its maximum is 100, when a species is present only in one group. Percent cover values are expressed as mean±SE. The R (reaction) value (range 1–9), according to Ellenberg et al. (1991), indicates the realized ecological niche of the species in Central Europe. Low values indicate that species are typical for acidic soils, high values that they occur typically on base-rich soils. Landolt (1977) assigns a medium R value to *Carex sempervirens*, which is equivalent to an R value of 5 according to the Ellenberg system. All indicator values are statistically significant at $P < 0.05$.

Treatment and indicator plant species	IndVal	Percent cover		No. plots containing species		R-value
		Unlimed	Limed	Unl.	Lim.	
Liming						
Carex sempervirens	59	1.5±0.40	2.6±0.34	20	36	7
Leontodon hispidus	54	1.8±0.30	2.8±0.35	26	36	7
Lotus corniculatus	51	1.2±0.23	2.1±0.27	23	33	7
Bartsia alpina	50	0.8±0.12	1.1±0.12	26	35	7
Alchemilla vulgaris	44	0.7±0.16	1.2±0.21	17	28	-
Viola calcarata	27	0.1±0.05	0.5±0.15	7	15	8
Anthyllis v. ssp. alp.	21	0.0±0.03	0.3±0.12	1	9	8
Selaginella selagin.	20	0.0	0.1±0.03	0	8	7
No liming						
Gentiana purpurea	58	4.3±0.52	1.9±0.31	37	31	3
Campanula barbata	54	2.9±0.31	1.5±0.21	35	28	1
Arnica montana	54	5.1±0.50	3.2±0.46	37	32	3
Trifolium alpinum	28	2.4±0.76	0.4±0.18	15	8	2
Vaccinium uligino.	18	0.4±0.17	0.1±0.06	9	2	1

The composition of the soil microbial community was significantly altered by liming, but not by fertilization (Table 3-1: Soil microbial community). When soil pH was included in the redundancy analysis as a covariate, the effect of liming was no longer significant ($F_{1,68} = 1.4$, $P = 0.171$), suggesting that it was mediated by soil pH. Indicator species analysis showed that the three PLFAs 10Me16:0, br17:0, and 10Me17:0 were indicator PLFAs for unlimed plots (Table 3-3). Bacterial

Table 3-3. Indicator phospholipid fatty acids (PLFAs) for no-liming treatments revealed by indicator species analysis (Dufrene and Legendre 1997) and their concentration in limed and unlimed plots. PLFA concentrations are given in nmol/g dry soil ± SE.

PLFA	No liming	Liming	*P*
10Me16:0	8.5 ± 0.43	7.0 ± 0.33	0.017
br17:0	0.5 ± 0.02	0.4 ± 0.02	0.024
10Me17:0	2.1 ± 0.11	1.7 ± 0.09	0.040

biomass, fungal biomass, and AM fungal biomass did not differ between treatments.

Discussion

The experiment at the Schynige Platte set up by W. Lüdi in the early 1930s offers a unique opportunity to assess the response of a grassland ecosystem to a short-term perturbation over a longer period of time than most other field experiments.

In contrast to other long-term studies in grasslands like the Park Grass and Cedar Creek experiments, where treatments were applied continuously over time (Dodd et al. 1994, Inouye and Tilman 1995, Wilson and Tilman 2002), the subset of the experimental plots analyzed in this study was last fertilized and limed in the early 1930s. Liming quickly increased the pH from 4.5–5 at the beginning of the experiment up to neutrality in 1945, and within a few years both treatments strongly changed the productivity and composition of the vegetation (Lüdi 1959). Then, with time, soil and plant communities are expected to asymptotically approach those of the control plots (Neubert and Caswell 1997). However, our results show that 70 years after the last treatment, the effects of liming on vegetation, soil microbial community, and on soil chemistry were still detectable.

Liming has generally strong effects on plant communities as shown during both short and long-term experiments, and these effects have been attributed to the change in soil pH (Tilman et al. 1994, Nilsson et al. 2002). At our study site, the pH of the limed plots remained close to neutrality until 1955, and then slowly returned to about pH 6.3 in 1990 (O. Hegg, unpublished data). In 2002, i.e., after 70 years, the pH of limed plots was still significantly higher than that of control plots. The difference was relatively small (5.14 vs. 4.97), but nevertheless represented a 50% higher concentration of hydrogen ions in the unlimed plots. We hypothesize that the current differences in plant community composition reflect the much larger differences in pH in the past, rather than the differences today. This may also explain why no persistent effects of liming on productivity could be detected in the greenhouse experiment. The plant community at the Schynige Platte consists mainly of long-lived, clonal perennials that should respond slowly to a gradual change in environmental conditions (Milchunas and Lauenroth 1995, Körner 1999).

In contrast, soil microorganisms are often short lived, have high turnover rates, and are thought to recover quickly from disturbance (Wardle 2002). Nevertheless, we found significant effects of liming on the composition of the PLFAs in the soil 70 years after the last treatment. This suggests that, despite the relatively small differences in soil pH and no evidence for differences in soil organic matter content and productivity, the limed plots continue to provide environmental conditions for belowground organisms that differ from those of the control plots. Experimental manipulation of the soil pH in forests resulted in consistent changes in PLFA patterns, suggesting that pH is

a key determinant of soil microbial community composition (Frostegård et al. 1993, Bååth et al. 1995, Pennanen et al. 1998, Bååth and Anderson 2003). Pennanen et al. (1998), in results similar to our study, found that even a small change in soil pH of 0.2 units significantly influenced the composition of the soil microbial community. In our study, both the results of the multivariate analysis and the response of individual PLFAs to liming indicated that the effects of the liming treatment were mediated by its effect on soil pH. In the experiment at the Schynige Platte, fatty acids that are typical for low pH (10Me16:0, 10Me17:0) were more abundant in unlimed plots (Frostegård et al. 1993, Pennanen et al. 1998, Bååth and Anderson 2003).

In regions like the Bernese Oberland where rainfall considerably exceeds evaporation, leaching of cations such as Ca is generally thought to be high due to significant water movement through the soil profile (Jenny 1980). However, the amount of exchangeable Ca in the soil of the limed plots, 70 years after the last liming, was still ~22% higher than in the control plots. Similarly, Marion et al. (1993) found that loss of exchangeable Ca from soils with a pH level comparable to that of our limed plots (pH ~7 in 1945) was slow in an arctic ecosystem. Based on a study along a primary successional chronosequence on an initially alkaline, Ca-rich soil in Alaska, Marion et al. (1993) estimated that it took 250 years until the amount of exchangeable Ca had declined by 75% and the soil pH level by 2–3 units.

A possible mechanism by which the long-term effects of liming can be explained is that the significant changes in soil pH originally induced by liming have subsequently been maintained by positive feedbacks

between belowground and aboveground ecosystem components (Wardle 2002). Soil pH is a key determinant of microbial community biomass and composition (Bååth and Anderson 2003), and has been shown to have a large influence on ecosystem processes such as litter decomposition (e.g., through its effect on degradation rates of phenolic compounds [Pind et al. 1994]) and nutrient mineralization. As a consequence, soils with a pH close to neutrality generally have a higher nutrient availability than soils with a pH of 4 to 5 (Chapin et al. 2002). Therefore, the changes in the belowground ecosystem induced by liming are likely to have favored plant species that have high rates of nutrient acquisition, high relative growth rates, and high tissue nutrient concentrations. Leaf litter of these plants is favorable to decomposers and breaks down more rapidly than that of plant species adapted to low-nutrient conditions (Cornelisson et al. 1999). The increased leaf litter quality in turn is likely to retain the soil microbial composition and the magnitude of microbial processes originally induced by liming. This feedback between aboveground and belowground food webs may generate relatively stable, mutualistic associations and thereby decrease the rate at which ecosystem components or processes return to their original state, or even shift the ecosystem to an alternative self-organized state.

Similar to the effects of liming, fertilization also resulted in a significant change in plant community composition and an increase in biomass production within the first years after application (Lüdi 1959). However, already in the early 1940s both biomass production and the abundance of plant species that had initially responded positively to fertilization began to decrease (Lüdi 1959). Correspondingly, we found only marginal effects of NPK fertilization on plant community

composition, and no effects on productivity and soil microbial composition. These results suggest that the subalpine acid grassland ecosystem at the Schynige Platte has a higher resilience to short-term fertilization than to short-term liming, and that the measured biotic and abiotic characteristics of the fertilized plots have again approached those of the control plots.

In summary, the results of this study provide strong evidence that short-term and small-scale perturbations can have very long-lasting effects on mountain grasslands, despite their high species richness, which is commonly seen as an indicator of high resilience (Hooper et al. 2005). The different long-term effects of liming and fertilization on soil chemistry and the composition of the plant and microbial community indicate that the resilience of mountain grasslands may differ depending on the type of ecosystem changes induced by the perturbation. We propose that the resilience of mountain ecosystems may be particularly low in response to perturbations that substantially alter soil pH or other key determinants of belowground processes.

Acknowledgements

We thank the Geschäftsleitung of the Alpengarten Schynige Platte for allowing us to work on the Lüdi plots, and Christine Alewell, two anonymous reviewers, and the subject matter editor for valuable comments on the manuscript. This work was supported by the Swiss Priority Program ‘‘Landscapes and Habitats of the Alps’’, a research project of the Swiss National Science Foundation (grant 4048-064424).

References

Bååth, E., and T. H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology & Biochemistry **35**:955-963.

Bååth, E., A. Frostegård, T. Pennanen, and H. Fritze. 1995. Microbial community structure and pH response in relation to soil organic-matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology & Biochemistry **27**:229-240.

Chapin III, F. S., P. A. Matson, and H. A. Mooney. 2002. Principles of Terrestrial Ecosystem Ecology. Springer Verlag, New York.

Cornelissen, J. H. C., N. Perez-Harguindeguy, S. Diaz, J. P. Grime, B. Marzano, M. Cabido, F. Vendramini, B. Cerabolini. 1999. Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytologist **143**: 191-200.

DeAngelis, D. L., S. M. Bartell, and A. L. Brenkert. 1989. Effects of nutrient recycling and food-chain length on resilience. American Naturalist **134**:778-805.

Dodd, M. E., J. Silvertown, K. McConway, J. Potts, and M. Crawley. 1994. Stability in the plant communities of the Park Grass Experiment: the relationships between species richness, soil pH and biomass availability. Philosophical Transactions of the Royal Society of London B **346**:185- 193.

Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs **67**:345-366.

Ellenberg, H., H. E. Weber, R. Düll, V. Wirth, W. Werner, and D.

Paulißen. 1991. Zeigerwerte von Pflanzen in Mitteleuropa, 3. edition. Goltze, Göttingen.

Frostegård, A., and E. Bååth. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils **22**:59-65.

Frostegård, A., E. Bååth, and A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biology & Biochemistry **25**:723-730.

Gunderson, L. H. 2000. Ecological resilience - in theory and application. Annual Review of Ecology and Systematics **31**:425-439.

Hedlund, K. 2002. Soil microbial community structure in relation to vegetation management on former agricultural land. Soil Biology & Biochemistry **34**:1299-1307.

Hegg, O., U. Feller, W. Dähler, and C. Scherrer. 1992. Long term influence of fertilization in a Nardetum. Vegetatio **103**:151-158.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual Review of Ecology and Systematics **4**:1-23.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer, D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. – Ecological Monographs **75**: 3-35.

Inouye, R. S., and D. Tilman. 1995. Convergence and divergence of old-field vegetation after 11 yr of nitrogen addition. Ecology **76**:1872-1887.

Jenny, H. 1980. The soil resource: origin and behavior. Springer Verlag, New York.

Johnston, A. E., K. W. T. Goulding, and P. R. Poulton. 1986. Soil acidification during more than 100 years under permanent grassland and woodland at Rothamsted. Soil Use and Management **2**:3-10.

Körner, C. 1999. Alpine plant life. Springer Verlag, Berlin - Heidelberg - New York.

Landolt, E. 1977. Ökologische Zeigerwerte zur Schweizer Flora. Stiftung Rübel, Zürich, Switzerland.

Lavorel, S., A.-H. Prieur-Richard, and K. Grigulis. 1999. Invasibility and diversity of plant communities: from pattern to processes. Diversity and Distributions **5**:41-49.

Leps, J., J. Osbornova-Kosinova, and M. Rejmanek. 1982. Community stability, complexity and species life-history strategies. Vegetatio **50**:53-36.

Leps, J., and P. Smilauer. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge.

Lüdi, W. 1948. Die Pflanzengesellschaften der Schynige Platte bei Interlaken und ihre Beziehung zur Umwelt. Veröffentlichung Geobotanisches Institut ETH, Stiftung Rübel **23**:400.

Lüdi, W. 1959. Versuche zur Alpweidenverbesserung auf der Schynigen Platte bei Interlaken. Alpengarten Schynige Platte, Interlaken.

MacGillivray, C. W., and J. P. Grime. 1995. Testing predictions of the resistance and resilience of vegetation subjected to extreme events. Functional Ecology **9**:640-649.

Marion, G. M., K. Cleve, and C. T. Dyrness. 1993. Calcium carbonate precipitation-dissolution along a forest primary successional sequence on the Tanana River floodplain, interior Alaska. Canada Journal of Forest Research **23**:923-927.

May, R. M. 1973. Stability and complexity in model ecosystems. Princeton University Press, Princeton, NJ, USA.

McCune, B., and M. J. Mefford. 1999. PC-ORD for Windows. Glenden Beach, USA.

Milchunas, D. G., and W. K. Lauenroth. 1995. Inertia in plant community structure: state changes after cessation of nutrient-enrichment stress. Ecological Applications **5**:452-458.

Moore, J. C., P. C. d. Ruiter, and H. W. Hunt. 1993. Influence of productivity on the stability of real and model ecosystems. Science **261**:906-908.

Mueller-Dombois, D., Ellenberg, H. 1974. Aims and methods of vegetation ecology. John Wiley & Sons, New York, USA.

Neubert, M. G., and H. Caswell. 1997. Alternatives to resilience for measuring the responses of ecological systems to perturbations. Ecology **78**:653-665.

Nilsson, M.-C., D. A. Wardle, O. Zackrisson, and A. Jäderlund. 2002. Effects of alleviation of ecological stresses on an alpine tundra community over an eight-year period. Oikos **97**:3-17.

Olsson, P. A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiology Ecology **29**:303-310.

Pennanen, T., H. Fritze, P. Vanhala, O. Kiikkila, S. Neuvonen, and E. Bååth. 1998. Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. Applied and Environmental Microbiology **64**:2173-2180.

Pind, A., C. Freeman, M. A. Lock. 1994. Enzymic degradation of phenolic materials in peatlands - measurement of phenol oxidase activity. Plant and Soil **159**: 227-231.

Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001.

Catastrophic shifts in ecosystems. Nature **413**:591-596.

Schimel, J. P., and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology **85**:591-602.

ter Braak, C. F., and P. Smilauer. 1999. Canoco for Windows. Center for Biometry, Wageningen, the Netherlands.

The R Development Core Team. 2004. R: A language and environment for statistical computing.

Tilman, D., M. E. Dodd, J. Silvertown, P. R. Poulton, A. E. Johnston, and M. J. Crawley. 1994. The Park Grass Experiment: insights from the most long-term ecological study. Pages 287-303 *in* R. A. Leigh and A. E. Johnston, editors. Long-term experiments in agricultural and ecological sciences. CABI, Wallingford.

Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. Nature **367**:363-365.

Tilman, D., and C. Lehman. 2001. Human-caused environmental change: impacts on plant diversity and evolution. Proceedings of the National Academy of Sciences (USA) **98**:5433-5440.

van den Wollenberg, A. L. 1977. Redundancy analysis: an alternative for canonical correlation analysis. Psychometrika **42**:207–219.

Wardle, D. A. 2002. Communities and ecosystems - linking the aboveground and belowground components. Princeton University Press, Princeton, NJ, USA.

Wilson, S. D., and D. Tilman. 2002. Quadratic variation in old-field species richness along gradients of disturbance and nitrogen. Ecology **83**:492-504.

Sawdust addition as a promising tool to reduce productivity of nitrogen enriched mountain grasslands

Thomas Spiegelberger, Diethart Matthies, Heinz Müller-Schärer and Urs Schaffner

Paper submitted

Abstract

Anthropogenic nutrient enrichment is threatening the high plant species richness of mountain grasslands. Grasses and fast-growing unpalatable plants are likely to profit over-proportionally from increased nutrient availability, thereby out-competing slow-growing species typical for species-rich mountain grasslands. A potential tool to reduce nutrient availability and above-ground productivity, and as a consequence promote plant diversity, is carbon addition to the soil. This method has been successfully applied to reduce interspecific competition among plant species in lowland grasslands. However, little is known whether this technique is also suitable for reducing productivity and altering vegetation composition of mountain grasslands. Here we report the results of a 3-year field study assessing the effects of sawdust addition on soil nutrients, above-ground productivity and vegetational composition of both grazed and ungrazed mountain grassland sites in Switzerland and France, with special emphasis on the performance of the tall unpalatable weed *Veratrum album*.

After three years, soil pH, soil inorganic nitrogen and plant-available phosphorus were not influenced by the sawdust treatment. However, biomass of the matrix vegetation (vegetation without *V. album*) was about 25% lower in sawdust-amended plots, similarly for forbs and grasses, while the biomass of *V. album* was only marginally affected. The cover of the matrix vegetation and of grasses was significantly lower in sawdust treated plots. However, sawdust treatment did not affect the cover of *V. album* and of other forbs, and did not increase species richness or change vegetation evenness. The

results suggest that sawdust addition is a promising tool to decrease productivity of both grazed and ungrazed mountain grasslands within a relatively short period of time. However, this method does not seem to be effective in reducing the vigour of *V. album* and other fast-growing unpalatable plants with large below-ground organs. To restore the species richness of nutrient-enriched mountain grasslands, we suggest applying sawdust for more than three years and, where appropriate, combining it with seed addition.

Introduction

The availability of nutrients, in particular of nitrogen (N), is one of the most important determinants of vegetation composition. At the global scale, N deposition is seen as one of the five most important causes of biodiversity loss (Sala *et al.* 2000). Theoretical and experimental work suggests that above a certain level of primary productivity, species diversity declines (Suding *et al.* 2005). Given that human activity has doubled globally the amount of N available, and that critical loads for grasslands are exceeded in many parts in Europe (Holland *et al.* 2005), methods are needed to mitigate the potential loss of biodiversity and to counter the negative effects of nutrient enrichment on plant diversity.

The plant species richness of mountain grasslands is still high compared to that of many lowland areas (MacDonald *et al.* 2000). The vegetation is characterised by plants adapted to nutrient-poor conditions (Kammer & Möhl 2002) which are poor competitors at higher N levels (Pywell *et al.* 2003). Because N mineralisation rates and N uptake by plants are low in mountain grasslands (Körner 2003), even small amounts of additional inorganic N may substantially decrease the species richness of mountain grasslands. Fertilisation and intensive grazing of mountain grasslands have been shown to reduce species richness and to favour grasses which are often negatively correlated with plant species richness in mountain grasslands (Oksanen, 1990; Theodose & Bowman, 1997; chapter 2). Apart from grasses, fast-growing unpalatable forbs, such as *Veratrum album* L., *Gentiana lutea* L. or *Cirsium eriophorum* L. are also likely to profit from increased nutrient availability in mountain grasslands. They

have been found to decrease species richness in their vicinity (Kleijn & Müller-Schärer, 2006; chapter 2).

Thus, the successful restoration of former species-rich mountain grasslands that are currently dominated by grasses and unpalatable plants must be based on a reduction of nutrient availability to levels comparable to that of traditional extensively managed grasslands. Most restoration techniques currently applied in lowland areas to reduce nutrient availability, such as top soil removal or maximising off-take of above-ground biomass, are not adequate for mountain grasslands. Removal of the top soil would not only eliminate the perennial vegetation and the soil seed bank, but could also result in a complete loss of the fertile top soil layer and leave bare bedrock. Maximising biomass off-take over 25 years has been demonstrated not to change grasslands composition compared to grazing (Kahmen *et al.* 2002).

An alternative technique to lower plant available N levels is the addition of a labile carbon (C) source to the soil. It has been shown that C addition to the soil stimulates soil microbial activity, resulting in an increased immobilisation of inorganic N (Schmidt *et al.* 1997b; Blumenthal *et al.* 2003). In a greenhouse experiment, this method proved to affect plant growth in a species-specific way, mainly reducing the above-ground biomass of annual species and perennial grasses (Eschen *et al.* 2006a). Despite a general reduction of plant growth, C addition may specifically favour slow-growing species due to a release from competition for light (Blumenthal *et al.* 2003). Carbon addition has successfully been used to selectively influence the competitive interactions among native plant species during early

succession (Török *et al.* 2000; Eschen *et al.* 2006a), and between invasive and native species (Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Blumenthal *et al.* 2003). However, it has rarely been tested as a tool to alter the community composition of perennial native vegetation (Michelsen *et al.* 1999; Prober *et al.* 2005).

In nutrient-rich lowland habitats, where plant growth is thought to be primarily limited by the availability of inorganic soil N (Schimel & Bennett 2004), C amendments have been shown to immobilise inorganic soil N and to limit the growth of plants (Reever Morghan & Seastedt 1999; Corbin & D'Antonio 2004). However, there is growing evidence that plants of arctic and alpine habitats in particular can also take up considerable quantities of organic N (Hodge *et al.* 2001; Nordin *et al.* 2004), due to their symbiotic relationship with arbuscular mycorrhizal fungi (AMF; Blaschke 1999) which play a direct role in decomposition and organic nutrient uptake (Hodge *et al.* 2001; Schimel & Bennett 2004). Consequently, plants in alpine and arctic ecosystems may be less dependent of inorganic N than plants growing in habitats with milder climatic conditions. So far, little is known about the suitability of C addition as a tool to reduce nutrient availability and alter vegetation composition in artic and alpine grasslands. The two studies we are aware of either combined C addition with top-soil removal (McLendon & Redente 1991), or assessed its potential in the context of competitive interactions between two common plant species (Michelsen *et al.* 1999).

Here we report the results of a 3-year field study to quantify the effects of C addition applied as sawdust on soil inorganic nutrients, above-ground productivity and vegetation composition of mountain

grasslands. In particular, we were interested in assessing the response of grasses and of *Veratrum album* L., which was chosen as a model species for fast-growing, unpalatable pasture weeds. We hypothesise that sawdust addition would over-proportionally reduce the growth of grasses and of *V. album* and, as a consequence, promote the competitive ability of slow-growing species characteristic of mountain grasslands, ultimately leading to increased species richness.

Materials and methods

Study sites and treatments

In spring 2002 we selected ten sites in two mountain ranges (Swiss Jura and Alps) situated below the tree line (Table 4-1). All sites were heavily infested by *V. album* (Liliaceae). *Veratrum album* is a long-lived, unpalatable plant common in mountain grasslands, where it can reach high densities in underexploited or abandoned pastures. In Europe, *V. album* has attained pest status in France, Switzerland, Italy, Austria and Slovenia (FAO, unpublished report). *Veratrum album* is typically found in natural grasslands above the tree line and in open woodlands, but has invaded semi-natural grasslands below the tree line. Large herbivores do not browse on *V. album* due to the high alkaloid concentration in all plant parts. Ingestion by livestock can result in abortion, deformation of embryos or the death of the animals (Binns et al., 1972). A more detailed description of the ecology and current management of *V. album* is given by Schaffner *et al.* (2001) and Kleijn & Müller-Schärer (2006).

Table 4-1: Description of the study sites. Mean annual precipitation, mean annual temperature and days with frost were obtained from the nearest permanent weather station available (www.meteoswiss.ch and www.meteo.fr). CH, Switzerland; F, France; NA, data not available.

Site	Region	Elevation in m	Mean annual precipitation	Mean annual temperature	Days with frost
Col des Mosses	Alps, CH	1500	1366	6·1	140
Col du Joly	Alps, F	1750	1621	6·8	NA
Grenchenberg	Jura, CH	1370	1155	3·2	160
La Meuser	Jura, CH	1400	1155	3·2	160
La Petite Ronde	Jura, CH	1140	1274	5·3	133
La Zorinche	Alps, F	1470	1571	6·3	NA
Mont Crosin	Jura, CH	1170	1155	3·2	160
Mungbach	Alps, CH	1380	1249	6·1	123
Tremont	Alps, CH	1480	982	2·0	183
Vieille Mole	Jura, CH	1090	1406	5·8	151

At each site, we set up one plot of 6 x 3-m in a grazed and an ungrazed grassland. Grazed grasslands were used as pastures for dairy cattle or young heifers. Ungrazed grasslands had been formerly used as pastures and were abandoned 1 - 20 years before the beginning of the experiment. In a few cases farmers indicated that the cattle might be driven across the ungrazed grasslands; therefore, the plots in the ungrazed grasslands were fenced with barbed wire at the start of the experiment to prevent any damage by cattle grazing or trampling. The maximum distance between the two paired plots was 1 km. All study sites were dominated by grasses; legumes represented approximately 10% of the cover of all forbs, and 5% of the biomass of all forbs.

Each plot consisted of two subplots (3 x 3-m). From 2002 to 2004, air-dried sawdust was applied by hand to one of the subplots monthly

at a rate of 0.5 kg/m² per application during three subsequent months (resulting in a total of 1.5 kg/m²yr), starting immediately after snow melt, except for the first year, when applications started in June. Sawdust was obtained from a local sawmill and consisted of wood from deciduous trees only. The other subplot was used as control.

Soil, biomass and vegetation sampling

Twelve soil samples (ø 2.5 cm, depth 10 cm) were taken in the outer 0.5 m of each subplot in autumn 2005 and pooled per subplot. They were transferred to a deep-freezer (-18 °C) within a few hours after sampling and kept frozen until further processing. $NH4^+$ and $NO3^-$ concentrations were measured with a colorimeter (Flow analyser, Skalar San Plus, The Netherlands) after solving 50 g soil in 250 ml of 1 m KCl, and ortho-phosphate with the Olsen method (Olsen et al. 1954). Soil pH was measured after shaking 1 g soil in 5 ml distilled water for 15 min. All soil analyses were carried out by SADEF, Aspach, France.

The above-ground biomass was harvested in autumn 2004 at least six weeks after the cattle had left the pasture, and in spring 2005, before cattle started to graze. Above-ground biomass of all forbs (except for that of *V. album*) and that of grasses including dead standing biomass was clipped 1 cm above ground in two 0.2 x 0.5-m rectangles in the outer 0.5 m of each subplot and pooled per subplot to obtain the biomass of the two functional groups. These two functional groups together are subsequently called “matrix vegetation”. Biomass of the matrix vegetation was dried for 2 days at 80 °C and weighted. In autumn 2004, when the above-ground parts of the plants had started to die back, the number of shoots of *V. album* in the central 2 x 2-m

square of each subplot was counted and the plants harvested above-ground, dried for 2 days at 80 °C and weighted. Biomass per shoot was calculated as the total above-ground biomass per plot divided by the number of shoots per plot. Three plots had to be excluded from further analysis because farmers had already cut the shoots of *V. album*. For comparisons with the matrix vegetation the biomass of *V. album* was corrected for the larger sampling area used. To obtain the total biomass per plot we summed up the corrected biomass of *V. album* and the biomass of the matrix vegetation in autumn 2004.

In each subplot, the vegetation of the central 2 x 2-m square was censused in summer 2002 and in spring 2005. The foliar cover of each plant species and the area covered by bare ground and rocks were visually estimated to the nearest percent. Species with a cover of < 1% were assigned a cover value of 0.5%. The cover of all forbs (except for that of *V. album*) and that of grasses was pooled to obtain the total cover of the matrix vegetation. Cover data from summer 2002 were used to assess possible initial differences in vegetation cover among treatments. The results indicated that at the beginning of the experiment, total vegetation cover, cover of *V. album*, of grasses, and of forbs did not consistently differ between the control plots and those plots that subsequently received sawdust addition across all experimental sites (all $P > 0.1$).

Statistical analysis

Cover data were used to calculate the Shannon-Wiener index of diversity ($H = - \sum pi \ln pi$, where pi is the relative abundance of species i), and evenness ($H/Hmax = H/\log S$, where S is the species richness). The mean Ellenberg light indicator value (mL, Ellenberg et al. 1991)

was calculated for each 1-m² plot as mL = ∑ Li x pi, where Li is the light indicator value of species i and pi its relative abundance. Mean Ellenberg nitrogen (mN), humidity (mH) and reaction indicator values (mR) were computed analogously.

All univariate data were analysed by linear mixed effect models with grazing and C treatment as fixed factors and site and plot as spatial random effects. Site and grazing was tested against plot, C treatment and its interaction with grazing against subplots. Vegetation data were log-transformed prior to analysis. Differences in vegetational composition were analysed by canonical correspondence analysis (CCA) using CANOCO 4.02 (ter Braak & Smilauer, 1999). The significance of the treatment effects was tested using the randomization test available in CANOCO. All other analyses were carried out with SPSS for Windows V.10.

Results

Soil variables

Sawdust application did neither influence soil pH (5.8 ± 0.15 for control vs. 5.9 ± 0.14 for sawdust treatment) nor the concentrations of $NH4^+$ (48.8 ± 6.52 vs. 43.5 ± 4.65), $NO3^-$ (1.4 ± 0.35 vs. 2.7 ± 1.01) or ortho-phosphate (11.4 ± 1.56 vs. 11.8 ± 1.46) (all $P > 0.05$).

Above-ground biomass

In autumn 2004, total above-ground biomass was 14% and above-ground biomass of the matrix vegetation 20% lower in the sawdust-amended than in the control plots (Table 4-2). Similarly,

above-ground biomass of the matrix vegetation in spring 2005 was about 25% lower in sawdust-amended than in control plots. The biomass of grasses and that of forbs were affected to a similar extent (4-2). In contrast, total biomass of *V. album*, number of shoots and biomass per shoot of *V. album* were not significantly influenced by C addition at the end of the third year (P > 0.1).third year (*P* > 0.1).

In autumn 2004, grazed plots had c. 30% less total ($F_{1,7}$ = 12.4, P = 0.010) and less matrix biomass ($F_{1,9}$ = 10.5, P = 0.010) and in spring 2005 about 40% less grass biomass (18 ± 2.59 g vs. 30 ± 2.88 g; $F_{1,9}$ = 9.3, P = 0.014) and about 35% less forb biomass (11 ± 1.28 g vs. 17 ± 1.53 g; F1,9 = 7.2, P = 0.025) than ungrazed plots, probably because harvested biomass of ungrazed plots contained more dead standing biomass compared to grazed plots. There was no interaction between the effects of the sawdust treatment and that of grazing, neither in autumn 2004, nor in spring 2005 (both P > 0.5). Species richness in spring 2005 decreased significantly with total above-ground biomass in autumn 2004 (r^2 = 0.28, $F_{1,32}$ = 6.0, P = 0.001; Fig. 4-1), and was also negatively correlated with above-ground biomass of the matrix vegetation in spring 2005 (r^2 = 0.08, $F_{1,38}$ = 3.4, P = 0.074).

Table 4-2: (a) Mean cover (± 1 SE), (b) biomass (± 1 SE) in autumn 2004 and (c) biomass (±1 SE) in spring 2005 as influenced by the addition of sawdust in the field. ns, P > 0.1; °, P > 0.05; *, P < 0.05: **. P < 0.01.

	a) Cover in % (spring 2005)			b) Biomass in gr (autumn 2004)			c) Biomass in gr (spring 2005)		
Functional group	Control	Saw-dust		Control	Saw-dust		Control	Saw-dust	
Total cover	104±3	100±3	°	59±4.9	51±4.3	°	---------	---------	
Matrix	93±3.1	87±3.0	*	50±4.2	40±3.8	°	43±3.9	32±3.4	**
Grasses	50±1.5	45±2.0	*	---------	---------		29±3.0	21±2.4	*
Forbs	43±2.9	41±2.8	ns	---------	---------		15±1.6	11±1.5	*
V. album	11±2.0	13±2.1	ns	7±1.0	8±1.0	°	---------	---------	

Vegetation cover and composition in 2005

In spring 2005, the cover of the matrix vegetation was significantly lower in the sawdust-amended plots than in the control plots, whereas total vegetation cover was only marginally reduced (Table 4-2). The lower cover of the matrix vegetation could be mainly attributed to a significant reduction in the cover of grasses by about 5% in sawdust-amended plots, while neither the cover of forbs nor of *V. album* was affected. Abandonment of grazing significantly reduced cover of grasses (45 ± 1.7% vs. 50 ± 1.8%; $F_{1,9}$ = 5.3; P = 0.047) and led to a different vegetation composition (CCA, $F_{1,9}$ = 1.5; P = 0.004), but did not interact with the sawdust treatment (for both P > 0.2). The decrease in grass cover by sawdust addition did not change the overall vegetational composition in spring 2005 (CCA, $F_{1,18}$ < 0.9; P > 0.8), nor did it increase the proportion of bare ground ($F_{1,18}$ < 1.2; P > 0.2). Moreover, the sawdust treatment did not change species richness, Shannon-diversity, evenness, or the mean Ellenberg indicator values (all P > 0.4).

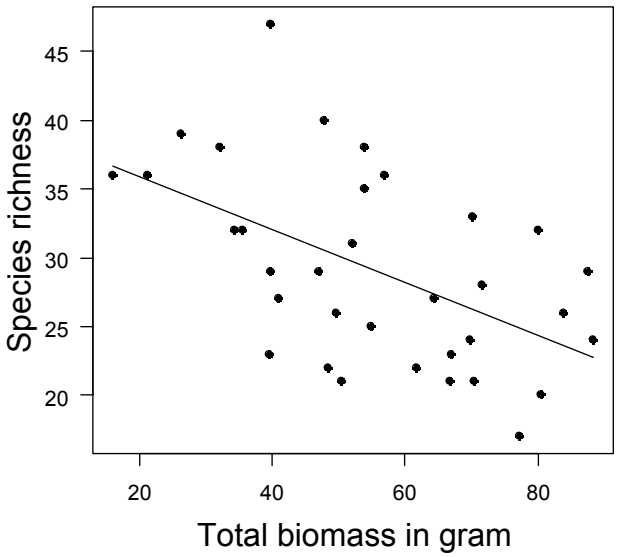


Fig. 4-1: The relationship between the species richness in spring 2005 and the total biomass in autumn 2004 for all plots of the experiment.

Discussion

To our knowledge, this study provides the first evidence that C addition can decrease the above-ground productivity of mountain grasslands without disturbing the soil or the perennial vegetation. After three years of consecutive application of sawdust, the cover of one of our target groups, the grasses, but also the biomass of grasses and forbs were significantly lower in the sawdust-amended plots than in the control plots. In contrast to Michelsen et al. (1999), who studied the effect of sugar on two common species in arctic grasslands, we applied a less easily degradable and cheaper form of carbon (sawdust). Moreover, in contrast to a previous study on a mountain grassland in which the top-soil was removed prior to carbon addition (McLendon & Redente 1992), we applied sawdust directly on the perennial vegetation. The results indicate that sawdust addition is a promising tool to reduce the above-ground productivity of both grazed and ungrazed mountain grasslands. Because our multi-site study covered a wide range of different soil and climatic conditions (Table 4-1), the results may indeed allow generalisation of our findings.

While three years of sawdust addition significantly reduced above-ground productivity, soil inorganic N levels did not vary between C amended and control plots. A possible explanation for the lack of response of soil inorganic N levels to sawdust addition in our study is that in arctic and alpine ecosystems, in contrast to many other ecosystems, a high proportion of nutrients is fixed in soil organic matter. Nutrients are only slowly released because of environmental constraints on decomposition, paired with high nutrient immobilisation by soil micro-organisms (Jonasson et al. 1996; Bowman et al. 2006).

Maximal plant nutrient uptake in cold climate grasslands occurs normally immediately after snow melt when nutrients are leached from the microbial biomass (Schimel & Mikan 2005). However, our soil samples were taken in late autumn (together with the above-ground biomass), when N concentrations measured as $NO3^{+}$ or $NH4^{-}$ were low. Moreover, in arctic and alpine ecosystems with low amounts of inorganic N, plants and microbes probably compete primarily for organic N, which we did not measure. Organic N, such as in amino-acids, is considered to be a significant N source for many herbaceous plants of arctic and alpine ecosystems which form a symbiotic relationship with AMF (Schimel & Bennett 2004). In a greenhouse experiment in which we grew two grass species on soils from our study sites, a reduction of AMF infestation through application of a fungicide resulted in a 30% decrease in plant productivity on sawdust amended soil, while productivity remained the same on soils collected from control plots (chapter 5), indicating that AMF are an important support system buffering at least to some extent a shortage in nutrient availability.

It could be argued that the significant effect of sawdust addition on productivity might be mediated by an at least temporarily shading of the soil surface and partially of the vegetation through the application of sawdust. However, in the same greenhouse experiment, during which no additional C amendment was made, the productivity of the two grasses grown on soil collected from C amended plots was also reduced (chapter 5). This suggests that the lower productivity of the C amended plots in the field experiment was indeed the result of a change in soil processes mediated by sawdust addition.

The reduction in above-ground biomass of the matrix vegetation in response to sawdust addition was paralleled by a change in the cover of functional groups. While the biomass of grasses and forbs were affected to a similar degree, the cover of grasses was more strongly reduced by sawdust addition than that of forbs. Previous field studies (Michelsen et al. 1999; Eschen et al. 2006b) and greenhouse studies (Schmidt et al. 1997a; Eschen et al. 2006a) have also found that cover or biomass of grasses was significantly reduced by soil C addition, while that of forbs remained unaffected or even increased. A possible explanation for the stronger reaction of grasses to the C treatment is that grasses react rapidly to changes in nutrient availability by an increased allocation of resources to below-ground growth (Schmidt et al. 1997a; Eschen et al. 2006a).

Studies in sub-alpine (Antonsen & Olsson 2005) and alpine grasslands (Heer & Körner 2002) revealed that graminoids profit most from elevated nutrient availability and increase in both, productivity and cover if fertilised. Additionally, in the comparative study in mountain grasslands (chapter 2), we found a significant negative correlation between grass cover and plant species richness. Species richness peaked at a grass cover of about 40%, while species richness was lowest when grass cover was 50% or more (chapter 2). These results indicate that C addition may indeed be a promising tool to increase species richness of nutrient-enriched mountain grasslands by reducing overall above-ground productivity and cover of grasses. Three years of sawdust application reduced the grass cover from 50% to 45%. Yet, neither species richness nor evenness of the vegetation did change over the three-year study period. Most alpine plants are long-lived plant species that have developed mechanisms allowing

them to persist even under high nutrient stress (Körner, 2003). Nevertheless, we suppose that in the long-term sawdust addition will increase species richness by releasing slow-growing plant species from above-ground competition through an over-proportional reduction of the grass cover (Blumenthal et al. 2003). Experimental evidence suggests, though, that plant species richness in mountain grasslands is not only limited by interspecific competition for light, but also by seed availability (Eskelinen & Virtanen 2005). Additional seed supply through hay transfer has been shown to increase species richness (Patzelt et al. 2001) and this could be an ideal complement to C addition.

Contrary to our predictions, the unpalatable weed *V. album* was not affected by the addition of sawdust during our study period. C addition has been used repeatedly as a management tool to restore grasslands that had been enriched in nutrients and invaded by exotic weeds. In most of these studies, the cover or productivity of the exotic weeds was successfully reduced (e. g. Reever Morghan & Seasedt 1999; Alpert & Maron 2000; Blumenthal et al. 2003; Prober et al. 2005). The apparent lack of response of *V. album* to sawdust addition may be due to its large below-ground storage organ which may act as a buffer against reduced nutrient availability. Approximately 60% of the N allocated to shoot growth of *V. album* at peak biomass originates from stored reserves (Kleijn et al. 2005) indicating a significant capacity of *V. album* to store N in below-ground organs. As a consequence, *V. album* is likely to respond only slowly, if at all, to changes in soil nutrient supply. We expect that other tall weeds with large underground storage organs like *Gentiana lutea* or *Cirsium eriophorum* may show a similar response. Therefore, decreasing the

above-ground biomass of such extensification weeds is not feasible with C addition alone, at least not in the short-term. Early cutting and prevention of seed production of these unpalatable weeds may be a more efficient method for their control (Kleijn et al. 2005; Hesse et al. 2006).

Conclusions

Our experiment provides first evidence that sawdust addition is a potentially useful method, and applicable without previous soil treatment, to reduce or mitigate negative effects of anthropogenetic nutrient enrichment on mountain grasslands. The relatively quick response of the vegetation to C addition is remarkable, because mountain ecosystems are characterized by low nutrient turnover rates and a slow recovery from changes in nutrient availability (chapter 3). Three years of sawdust addition reduced the above-ground biomass of the matrix vegetation, but did not (yet) increase species richness, although species richness increased with decreasing biomass at our sites. The vegetation of mountain grasslands consists almost completely of long-lived plant species (Matejkova et al. 2003) and increases in species richness may, thus, need more time. Long-term research is necessary to (i) further study the effect of sawdust amendment on species richness of nutrient-enriched mountain grasslands, in combination with seed addition and specific control measures for unpalatable plants with large below-ground storage organs, and (ii) to assess the sustainability of these measures under various land-use scenarios.

Acknowledgement

This work was funded by the Swiss Priority Program (NFP 48) “Landscapes and Habitats of the Alps”, a research project of the Swiss National Science Foundation (grant 4048-064424 to HMS and US). We are grateful to all landowners who allowed us to establish experimental plots in their pastures.

References

Alpert, P. & Maron, J.L. (2000) Carbon addition as a countermeasure against biological invasion by plants. *Biological Invasions*, **2**, 33-40.

Antonsen, H. & Olsson, P.A. (2005) Relative importance of burning, mowing and species translocation in the restoration of a former boreal hayfield: responses of plant diversity and the microbial community. *Journal of Applied Ecology*, **42**, 337-347.

Binns, W., Keeler, R.F., & Balls, L.D. (1972) Congenital deformation in lambs, calves and goats resulting from maternal ingestion of *Veratrum californicum*: hare clip, cleft palate, ataxia and hypoplasis of metacarpal and metatarsal bones. *Clinical Toxicology*, **5**, 245-261.

Blaschke, H. (1991) Distribution, mycorrhizal infection, and structure of roots of calcicole floral elements at treeline, Bavarian Alps, Germany. *Arctic, Antarctic, and Alpine Research*, **23**, 444-450.

Blumenthal, D.M., Jordan, N.R., & Russelle, M.P. (2003) Soil carbon addition controls weeds and facilitates prairie restoration. *Ecological Applications*, **13**, 605-615.

Bowman, W.D., Gartner, J.R., Holland, K. & Wiedermann, M. (2006) Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: are we there yet? *Ecological Applications*, **16**, 1183-1193.

Corbin, J.D. & D'Antonio, C.M. (2004) Can carbon addition increase competitiveness of native grasses? A case study from California. *Restoration Ecology*, **12**, 36-43.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., & Paulißen, D. (1991) Zeigerwerte von Pflanzen in Mitteleuropa, 3. edn. Goltze, Göttingen.

Eschen, R., Müller Schärer, H., & Schaffner, U. (2006a) Soil carbon addition affects plant growth in a species-specific way. *Journal of Applied Ecology*, **43**, 5-42.

Eschen, R., Mortimer, S.R., Lawson, C.S., Edwards, A.R., Brook, A.J., Igual, J.M., Hedlund, K., & Schaffner, U. (2006b) Carbon addition alters early succession on ex-arable fields. *Journal of Applied Ecology*, in press.

Eskelinen, A. & Virtanen, R. (2005) Local and regional processes in low-productive mountain plant communities: the roles of seed and microsite limitation in relation to grazing. *Oikos*, **110**, 360-368.

Heer, C. & Körner, C. (2002) High elevation pioneer plants are sensitive to mineral nutrient addition. *Basic and Applied Ecology*, **3**, 39-47.

Hesse, E., Rees, M., Müller-Schärer, H. (2006) Seed bank persistence of clonal weeds in contrasting habitats: implications for control. *Plant Ecology*, in press.

Hodge, A., Campbell, C.D., & Fitter, A.H. (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, **413**, 297-299.

Jonasson, S., Michelsen, A., & Schmidt, I.K. (1999) Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. *Applied Soil Ecology*, **11**, 135-146.

Holland, E.A., Braswell, B.H., Sulzmann, J., Lamarque, J.F. (2005) Nitrogen deposition onto the United States and western Europe: Synthesis of observations and models. *Ecological Applications*, **15**, 38-57.

Kahmen, S., Poschlod, P., & Schreiber, K.F. (2002) Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. *Biological Conservation*, **104**, 319-328.

Kammer, P.M. & Möhl, A. (2002) Factors controlling species richness in alpine plant communities: an assessment of the importance of stress and disturbance. *Arctic, Antarctic, and Alpine Research*, **34**, 398–407.

Kleijn, D. & Müller-Schärer, H. (2006) The relation between unpalatable species, nutrients and plant species richness in Swiss montane pastures. *Biodiversity and Conservation*, in press.

Kleijn, D., Treier, U., & Müller-Schärer, H. (2005) The importance of nitrogen and carbohydrate storage for plant growth of the alpine herb *Veratrum album*. *New Phytologist*, **166**, 565-575.

Körner, C. (2003) *Alpine plant life*. Springer Verlag, Berlin - Heidelberg - New York.

MacDonald, D., Crabtree, J. R., Wiesinger, G., Dax, T., Stamou, N., Fleury, P.,

Gutierrez Lazapita, J. and Gibon, A. 2000. Agriculture abandonment in mountain areas of Europe: Environmental consequences and policy response. *Journal of Environmental Management*, **59**, 47-69.

Matejkova, T., van Diggelen, R., & Prach, K. (2003) An attempt to restore a central Europe species-rich mountain grassland through grazing. *Applied Vegetation Science*, **6**, 161-168.

McLendon, T. & Redente, E.F. (1992) Effects of nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. *Oecologia*, **91**, 312-317.

McLendon, T. & Redente, E.F. (1991) Nitrogen and phosphorus effects on secondary succession dynamics on a semi-arid sagebrush site. *Ecology*, **72**, 2016-2024.

Michelsen, A., Graglia, E., Schmidt, I.K., Jonasson, S., Sleep, D., & Quarmby, C. (1999) Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist*, **143**, 523-538.

Nordin, A., Schmidt, I.K., & Shaver, G.R. (2004) Nitrogen uptake by artic soil microbes and plants in relation to soil nitrogen supply. *Ecology*, **85**, 955-962.

Oksanen, J. (1990) Predation, herbivory, and plant strategies along gradients of primary production. *Perspectives on plant competition* (eds. J. Grace & D. Tilman), pp. 445-474. Academic Press, New York.

Olsen, S.R., Cole, C.V., Watanabe, F.S., & Dean, L.A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture Circulate 939.

Patzelt, A., Wild, U., & Pfadenhauer, J. (2001) Restoration of wet fen meadows by topsoil removal: Vegetation development and germination biology of fen species. *Restoration Ecology*, **9**, 127-136.

Prober, S.M., Thiele, K.R., Lunt, I.D., & Koen, T.B. (2005) Restoring ecological function in temperate grassy woodlands: manipulating soil

nutrients, exotic annuals and native perennial grasses through carbon supplements and spring burns. *Journal of Applied Ecology*, **42**, 1073-1085.

Pywell, R.F., Bullock, J.M., Roy, D.B., Warman, L.I.Z., Walker, K.J., & Rothery, P. (2003) Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology*, **40**, 65-77.

Reever Morghan , K.J. & Seastedt, T.R. (1999) Effects of soil nitrogen reduction on nonnative plants in restored grasslands. *Restoration Ecology*, **7**, 51-55.

Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfeld, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A.P., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, L., Sykes, M.T., Walker, B.H., Walker, M., & Wall, D.H. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770-1774.

Schaffner, U., Kleijn, D., Brown, V., & Müller Schärer, H. (2001) *Veratrum album* in montane grasslands: a model system for implementing biological control in land management practices for high biodiversity habitats. *Biocontrol News and Information*, **22**, 19n-28n.

Schimel, J.P. & Bennett, J. (2004) Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, **85**, 591-602.

Schimel, J.P. & Mikan, C. (2005) Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biology & Biochemistry*, **37**, 1411-1418.

Schmidt, I.K., Michelsen, A., & Jonasson, S. (1997a) Effect on plant production after addition of labile carbon to arctic/alpine soils. *Oecologia*, **112**, 305-313.

Schmidt, I.K., Michelsen, A., & Jonasson, S. (1997b) Effects of labile soil carbon on nutrient partitioning between an arctic graminoid and microbes. *Oecologia*, **112**, 557-565.

Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G., & Pennings, S. (2005) Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. *Proceedings of the National Academy of Science of the United States of America*, **102**, 4387-4392.

ter Braak, C.F. & Smilauer, P. (1999) *Canoco for Windows.* Wageningen.

Theodose, T.A. & Bowman, W.D. (1997) The influence of interspecific competition on the distribution of an alpine graminoid: evidence for the importance of plant competition in an extreme environment. *Oikos*, **79**, 101-114.

Tilman, D. (1997) Community invasibility, recruitment limitation, and grassland biodiversity. *Ecology*, **78**, 81-92.

Nutrient manipulation on mountain grassland – the importance of arbuscular mycorrhizal fungi

Thomas Spiegelberger and Urs Schaffner

Paper in preparation

Abstract

Mountain ecosystems harbour a high number of plants with the majority of species well adapted to nutrient poor conditions, making mountain ecosystems particularly sensitive to nitrogen (N) enrichment. A technique to at least temporarily lower plant available nutrients in grasslands is the addition of labile carbon (C) to the soil what reduces inorganic N and subsequently above-ground plant biomass. However, in cold climate ecosystems plants can also access organic N with the aid of arbuscular mycorrhizal fungi (AMF). Therefore a more mechanistic understanding of the effects of nutrient manipulations of mountain grasslands is needed. In a bioassay we tested whether sawdust decreased and N and/or phosphorus (P) fertilisation increased productivity of the phytometer species *Festuca rubra* and *Poa alpina* and whether grazed sites tend to be more N-limited and ungrazed sites more P-limited. Further, whether effects of C-amendments on productivity of *F. rubra,* which is characterised by high AMF-colonisation rates would be stronger in the absence than in the presence of AMF compared to *P. alpina* which tends to have lower AMF-colonisation rates.

Biomass productivity of plants was lowest after C-addition, followed by control, and highest after N and P-fertilisation. While *P. alpina* shown no reaction to fungicide application, *F. rubra* produced about 20 % (N) and about 30 % (C) less biomass when benomyl was applied. *P. alpina* which in the absence of AMF must rely for nutrient supply on inorganic N, produced nearly twice as much biomass on N-amended soil from grazed plots compared to ungrazed soil, while when P-fertilised, plants produced about 30 % more biomass on soil

form ungrazed plots compared to grazed plots. Soil analyses showed a higher N:P ratio on the ungrazed compared to the grazed sites suggesting a stronger N-limitation on grazed sites, but no effects of nutrient manipulation treatments.

The study provides evidence that N or P can be the limiting resource on mountain grasslands depending on land use form, and that sawdust addition is a promising tool to reduce productivity. As soil micro-organisms are at least temporally better competitors for plant available nutrients than plants, C-addition may decrease primarily of plants having low AMF-colonisation rates.

Introduction

In Europe, traditionally managed mountain grasslands harbour high plant species richness. However, predictions forecast that nitrogen (N) deposition will be a major driver of diversity loss in alpine regions (Sala et al. 2000), as above a certain level of primary production, species diversity declines in response to N enrichment (Gough et al. 2000, Gross et al. 2000, Suding et al. 2005). The vast majority of plant species growing in mountain ecosystems are characterised by traits that promote tolerance of low nutrient availability and low nutrient turn-over, making mountain ecosystems particularly sensitive to N-enrichment (Körner 1999). A more mechanistic understanding is therefore needed to developed management methods that aim to minimize the negative effects of N-enrichment on biodiversity on mountain grasslands.

Management strategies that are being employed to mitigate the effect of N-enrichment on grassland biodiversity in the lowland are not easily applicable in mountain ecosystems. Extensification or depletion of highly nutrient rich sites by means of top soil removal is often complicated and expensive in less accessible mountain grasslands, and would results complete elimination of the perennial vegetation and the fertile soil. Continuous biomass removal over 25 years has no significant effect on grasslands composition, compared to grazing (Kahmen et al. 2002).

Another technique to at least temporarily lower plant available nutrient levels in grasslands is the addition of a labile carbon (C) source to the soil. Based on the assumption that soil microbial

communities are primarily C-limited, and that plants can only use that part of the soil nutrients which exceeds the microbial demand (Blumenthal et al. 2003), it has been hypothesized that C-addition induces the resident soil bacteria and fungi to immobilize plant available soil nutrients (Schmidt et al. 1997b, Blumenthal et al. 2003). Indeed, already addition of less than 5% of the total C-content of a soil strongly increased the total microbial biomass under controlled conditions (Fontaine et al. 2004), and similar results were also obtained under field conditions (Jonasson et al. 1996, Schmidt et al. 2000). Moreover, microcosm and field studies in lowland ecosystems have shown that C-addition may induce a decrease in inorganic N-concentration in the soil (Schmidt et al. 1997b, Török et al. 2000), a reduction in above-ground vegetation biomass (Michelsen et al. 1999, Alpert and Maron 2000, Blumenthal et al. 2003) and a shift in the vegetation composition (Blumenthal et al. 2003, Eschen et al. 2006a).

However, it is becoming more and more apparent that the basic assumption that plants are inherently poor competitors for available soil N relative to soil microorganisms may be invalid (Schimel and Bennett 2004). In low-N ecosystems such as alpine or arctic ecosystems, organic N such as amino acids may be a significant N source for many plant species (Lipson and Monson 1998, McKane et al. 2002, Nordin et al. 2004). The uptake of organic N may be enhanced by arbuscular mycorrhizal fungi (AMF) which may also directly decompose organic matter (Schimel and Bennett 2004). The majority of herbaceous plants in arctic and alpine ecosystems form symbiotic relationships with AMF (Read and Haselwandter 1981, Blaschke 1991). Therefore, it is not evident whether C-addition to the soil is indeed likely to affect above-ground productivity and to induce a

shift in the vegetation composition. The only studies testing the effects of soil C-addition in high altitude ecosystems either applied sugar (Michelsen et al. 1999), a form of C-addition which is from an economical point of view not feasible, because too expensive, or removed the topsoil prior to C-treatments (McLendon and Redente 1992).

To evaluate whether soil C-addition affects productivity in mountain grasslands, we grew in a greenhouse experiment phytometer species on soil from experimental plots in grazed and ungrazed grasslands that had been amended with sawdust for three years. Also, phytometer plants were grown on soil that had been fertilized with either N or phosphorus (P) to assess the limiting nutrient on grazed and ungrazed mountain grasslands. As the association with mycorrhizae is considered to be a key mechanism by which plants can increase their mineral-nutrient uptake and access organic nutrients (Schimel and Bennett 2004), we added to half of the pots a fungicide, benomyl, to examine whether this would increase the effect of the previous C, N and P amendments to the soil. We used as phytometer species *Festuca rubra* L., a species which is known to have high AMF colonization rates, and *Poa alpina* L., which tends to have low AMF colonization rates in mountain grasslands (Read and Haselwandter 1981, Tscherko et al. 2004).

Specifically, we hypothesise that C decreases and N and/or P-fertilisation increases productivity of phytometer species growing on soil from mountain grasslands. Since inorganic N levels tend to be lower on grazed or mown compared to abandoned grasslands (Knops and Tilman 2000, Zeller et al. 2000), we predicted that grazed sites

would tend to be more N-limited and ungrazed sites more P-limited. Finally, due to the generally higher AMF colonization rates of *F. rubra* compared to that of *P. alpina*, we predicted that the effect of soil C-amendments on productivity of *F. rubra* would be stronger in the absence than in the presence of mycorrhizae, while the response of *P. alpina* productivity to nutrient addition treatments would be less affected by the fungicide treatment.

Materials and method

Study sites, field treatments and soil sampling

The field nutrient manipulation study was carried on five sites each in the Swiss Jura and the Alps. Each site had two plots with different grazing managements: one plot was grazed with young cows or heifer, the other was left ungrazed. Each plot (6 m x 6 m) consisted of four subplots of 3 m x 3 m which were subjected to four treatments. From spring 2002 to autumn 2004, air-dried sawdust was brought out by hand at a rate of 1.5 kg m^{-2} to the C-addition subplots in three increments of 0.5 kg m^{-2} per application, immediately after the snow melted, except for 2002, where applications started in June. The other subplot was used as a control. Two further subplots were set up in spring 2003 and were subjected to application of N as ammonium nitrate at a ratio of 1 g m^{-2} in 2003 and 10 g m^{-2} in 2004, and P as Triple-Super-Phosphate at a ratio of 0.1 g m^{-2} in 2003 and 1 g m^{-2} in 2004 once a year, mid-June. Quantities of nutrient additions in 2003 were miscalculated and only one-tenth of quantities in 2004. Both fertilisers were obtained from a commercial source (Landor, Birsfelden), grounded and sprayed with 1.5 l of water. The S- and the

C-subplot received the same amount of water. For a more detailed description of study sites and treatments, see chapter 4.

Twelve soil samples (ø 2.5 cm, depth 10 cm) were taken from each subplot in autumn 2004 to analyse soil chemical properties, and four soil samples (ø 7 cm, depth 15 cm) were taken from each subplot for a phytometer experiment under controlled conditions in the greenhouse. Soil for the greenhouse experiment was kept dark and cool (2° C) until further processing. In spring 2005, the soil samples were pooled per subplots, homogenized and sieved (mesh size 5 mm). Soil samples for the soil chemical analyses were pooled per subplot, transferred to a deep-freezer (-18 °C) within a few hours after sampling and kept frozen until further processing. All except one soil samples were analysed in January 2006. NH_4 and NO_3 concentrations were measured with a colorimeter (Flow analyser, Skalar San Plus, The Netherlands) after solving 50 g soil in 250 ml of 1 m KCl, and ortho-P with the Olsen method. N:P ratio was calculated as the sum of nitrate and ammonium concentrations divided by the total P concentration. Soil pH was measured after shaking 1 ml soil in 5 ml distilled water (all analyses were carried out by SADEF, Aspach, France and by SolConseil, Changins, Switzerland).

Greenhouse experiment

To assess the productivity of the soil from the nutrient manipulation plots, a greenhouse experiment was set up in January 2005 using the two common grass species *Poa alpina* L. and *Festuca rubra* L. as phytometer species. Soil samples from each of the 80 subplots were filled into 8 plastic tubes (diameter 2 cm, c. 100 cm^3), which were tightly closed by a 55 µm gauze at the bottom, individually

placed in cups and randomly arranged on a greenhouse bench. Two tubes per subplot were randomly assigned to the four treatment combinations described below. Each treatment comprised a 2 x 2 factorial with phytometer species (*P. alpina* or *F. rubra*) and benomyl application (yes or no), resulting in a total of 640 tubes. One seedlings of each phytometer species, previously surface-sterilised and germinated on moist filter paper in a climate chamber (20 °C, 8/16h dark/light), was planted into each tube. Seedlings that died within 10 days after transplanting were replaced. Benomyl was applied as Benlate® the same day as the seedlings were planted at a rate of 0.25 mg benomyl g^{-1} soil applied in a 0.75g l^{-1} aqueous solution, a concentration which was similar to that of other studies (Fitter and Nichols 1988, Frank et al. 2003). The benomyl solution was poured on the soil surface of each tube. Mycorrhizal controls received the same quantity of tab water.

Benomyl has been shown to have minimal direct effects on plants in some studies (Paul et al. 1989), and is a recommended method for manipulating soil fungi in experiments with plants (Fitter and Nichols 1988, Smith et al. 2000). However, we acknowledge that benomyl kills a wide range of fungal types (West et al. 1993) and can have non-target effects on other soil micro-organisms (Van der Putten et al. 2001) as AMF are also involved in protection against root pathogens, provision of C sink strength to their host , improvement of host water relations including drought tolerance, mediation of pollutant effects, resulting often, but not always, in improved host plant growth and fitness (cf. Rillig 2004 and references therein). Nevertheless it provides a higher degree of realism than approaches using soil sterilisation, artificial growing media, and the use of one or only a few AMF species

obtained from a source other than the native community under study (Hartnett and Wilson 2002). A study comparing benomyl incorporation into soil, gamma-irradiation of soil, and the use of a myc(-) mutant showed that the use of benomyl appeared to be the most appropriate method currently available as mycorrhizal infection rates are well reduced and soil nutrient status only slightly affected (Kahiluoto et al. 2000). Benomyl may, however, lead to an underestimation of AM effectiveness because the control is not totally non-mycorrhizal (Kahiluoto et al. 2000).

To assess the level of mycorrhizal colonization of the two grass species and the efficacy of the benomyl treatment, we checked the mycorrhizal colonization rates of both species in subsamples of five plants each growing on soil taken from the control plots with and without benomyl treatment. Dry roots were soaked in tap water for half an hour, afterwards cleared with 10% KOH and fungal structures inside the roots were stained with trypan blue (Phillips and Hayman 1970). The percentage of root length colonized by mycorrhizae was estimated by a modified line intersection method (van der Heijden 2004), where 75 intersections per root sample were scored for the presence of AM fungal structures. Roots of *F. rubra* plants growing on natural soil from the control subplots of the field nutrient manipulation experiment had an average mycorrhizal colonization rate of about 15 %, while *P. alpina* roots from the same treatments had an infestation rate of 4 %. These findings are in agreement with the literature records indicating that *F. rubra* is a species frequently associated with AMF, while *P. alpina* is a species with low mycorrhizal colonization rates. Colonization rates of *F. rubra* and *P. alpina* on benomy-treated soil

was 4% and 2%, respectively, indicating that the addition of benomyl successfully reduced mycorrhizal colonization to very low levels.

During the whole period of the experiment, all plants regularly received the same amount of water. At day 7, 14, 30, and 45, number of leaves and the length of the longest leaf were measured in each tube. For simplicity we refer to the product of leaf length and leaf numbers as “leaf length”. After 46 days the seedlings were harvested as roots started to accumulate on the bottom. Aboveground biomass (here after called “biomass”) was harvested at ground level, dried for 48 hours at 80° C and weighed. For both variables, leaf length and biomass, the mean of the each subplot x species x benomyl combination was used for further analysis.

Statistical analysis

For each subplot x species x benomyl combination, growth rate was calculated as the slope of a linear model with leaf length as response variable and log-transformed time-values as explanatory variable. Tubes where plants were limited by water availability due to the small soil volume and high temperature in the greenhouse during the experiment were excluded from further analysis. To avoid arbitrary decisions, we excluded all combinations with negative growth rates or P-values bigger than 0.1 of the linear model for growth rate. This resulted in a total of 241 subplot x greenhouse treatments combinations. As growth rate was highly related to biomass (linear regression, $r > 0.67$, $P < 0.001$), only analyses of biomass are shown here.

To test for differences in nutrient status of the soil taken from the 80 subplots in the field, hierarchical linear mixed effects models were calculated with all main factors in the order region, management, and nutrient manipulation treatments as fixed factors. Subplot, plot and sites were used as nested spatial random effects. Region was tested against sites, management and the interaction region x management against plot, and nutrient manipulation treatment and its interactions with region and management against subplots. Nitrate and total inorganic N were ln-transformed to meet the assumption of normally distributed residuals and homogeneity of variances of the linear mixed effect model.

To assess the effect of the treatments on biomass, a full factorial, hierarchical linear mixed effects model was calculated including all main factors and interactions in the order region, grazing management, nutrient manipulation treatments, phytometer species and benomyl application as fixed factors. Subplot, plot and sites were used as spatial random effects nested into each other. Region was tested against sites, management and the interaction region x management against plot, nutrient manipulation treatment and its interactions with region and management against subplots and phytometer species and benomyl application against error terms. For the individual analyses of the two phytometer species, two linear mixed effect models similar to the above described were calculated, except for the factor phytometer species which was omitted and that only two and three-fold interactions were included. Biomass was ln-transformed to meet the assumption of normally distributed residuals and homogeneity of variances of the linear mixed effect model. All

analyses were carried out with R 2.1.1 statistical language and corresponding packages (The R Development Core Team 2003).

Results

Soil analyses

None of the soil variables measured was significantly affected by nutrient treatments applied in the field (for all F < 1.1; P >0.3). N:P-ratio was significantly higher at ungrazed sites (6.8± 0.62) compared to grazed sites (4.32±0.60), suggesting a stronger N-limitation on grazed sites ($F_{1,8}$ = 7.0, P = 0.030).

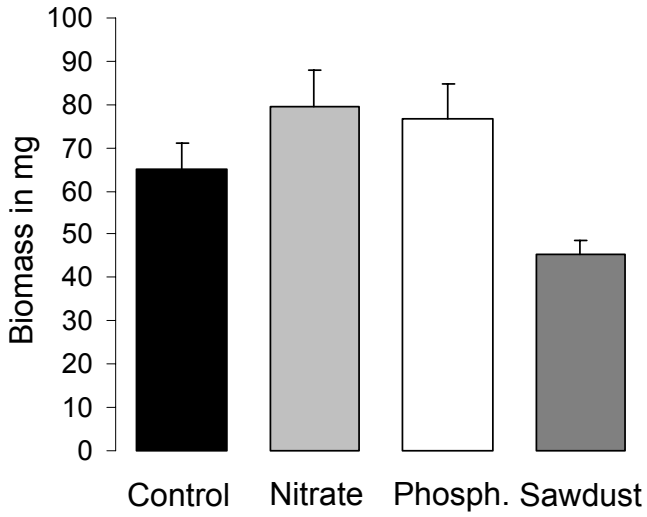


Fig. 5-1: Mean biomass in mg (+SE) of phytometer plants per nutrient manipulation treatment applied in the field.

Effects of field treatments

Nutrient manipulation treatments applied in the field significantly affected above-ground biomass of phytometer plants in the greenhouse ($F_{3,42}$ = 5.9, P = 0.002). Phytometer plants growing on sawdust amended soil produced less biomass compared to any other treatment (cf. Fig. 5-1), while plants on N or P amended plots had a higher biomass than plants on control plots. Above-ground biomass

was marginally affected grazing management ($F_{1,8}$ = 4.3, P = 0.071), and higher on grazed plots compared to ungrazed ones (74±4.9 vs. 60±5.2 mg).

Effects of greenhouse treatments

Phytometer species performed significantly different ($F_{1,8}$ = 5.5, P = 0.020). The main effect of benomyl application did not influence the biomass of phytometer plants (P > 0.3), but a marginal significant interaction between field treatments, species and benomyl application indicated ($F_{3,118}$ = 2.1, P = 0.099) different responses of phytometer plants to field treatments and benomyl application. We therefore performed two separate analyses for each phytometer species, which showed that *F. rubra* was marginally affected by the benomyl application, while *P. alpina* was not affected by the fungicide (cf. Table 5-1).In addition to the main effect of benomyl application, *F. rubra* was significantly affected by the interaction between field nutrient

Table 5-1: General linear model of the effects of region, grazing, field nutrient manipulations and benomyl application on biomass productivity of the two phytometer species *F. rubra* and *P. alpina* in a greenhouse experiment. Marginally significant p-values (0.1 >p >0.05) are in italics, significant p-values (p <0.05) are in bold-face.

	F. rubra				*P. alpina*		
	DF	denDF	F	P	denDF	F	P
Region (R)	1	8	4.2	*0.075*	7	1.2	0.306
Grazing (G)	1	8	5.2	*0.052*	7	1.9	0.216
Nutrients (N)	3	39	2.9	*0.050*	38	5.8	**0.002**
Benomyl (B)	1	35	3.0	*0.094*	41	0.3	0.617
R x G	1	8	0.2	0.692	7	0.0	0.995
R x N	3	39	0.8	0.505	38	0.5	0.707
G x N	3	39	1.3	0.291	38	3.0	**0.043**
R x B	1	35	7.1	**0.011**	41	0.1	0.816
G x B	1	35	0.9	0.358	41	0.1	0.766
N x B	3	35	2.9	**0.046**	41	0.7	0.551
R x G x N	3	39	1.7	0.182	38	1.6	0.200
R x G x B	1	35	3.4	*0.074*	41	0.1	0.783
R x N X B	3	35	0.3	0.839	41	1.2	0.330
G x N X B	3	35	0.6	0.607	41	0.2	0.916

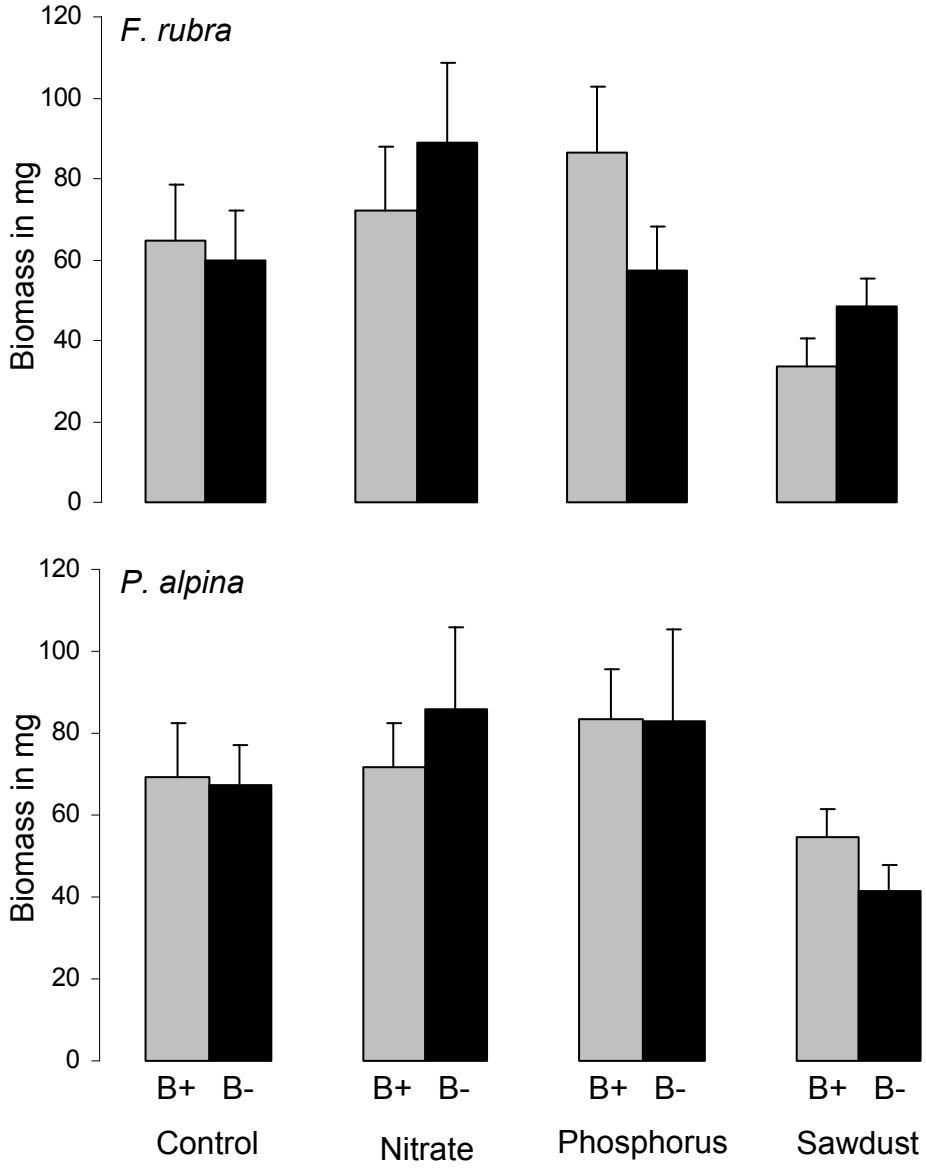


Fig. 5-2: Mean biomass in mg (+SE) of *F. rubra* (above) and *P. alpina* (below) phytometer plants per nutrient manipulation treatment and benomyl treatment (B+, benomyl application; B-, no benomyl application). For statistics see Table 5-1.

manipulation treatments and benomyl application (cf. Table 5-1 & Fig. 5-2). Plants growing on soil from control plots performed similarly, while on both, N and on sawdust amended plots, plants produced about 20 % (N) and about 30 % (sawdust) less biomass when benomyl was applied. Plants on P-fertilised soil reacted differently compared to plants on N and sawdust amended soils. In the latter case 33 % more biomass was produced when tubes were drenched with a benomyl solution.

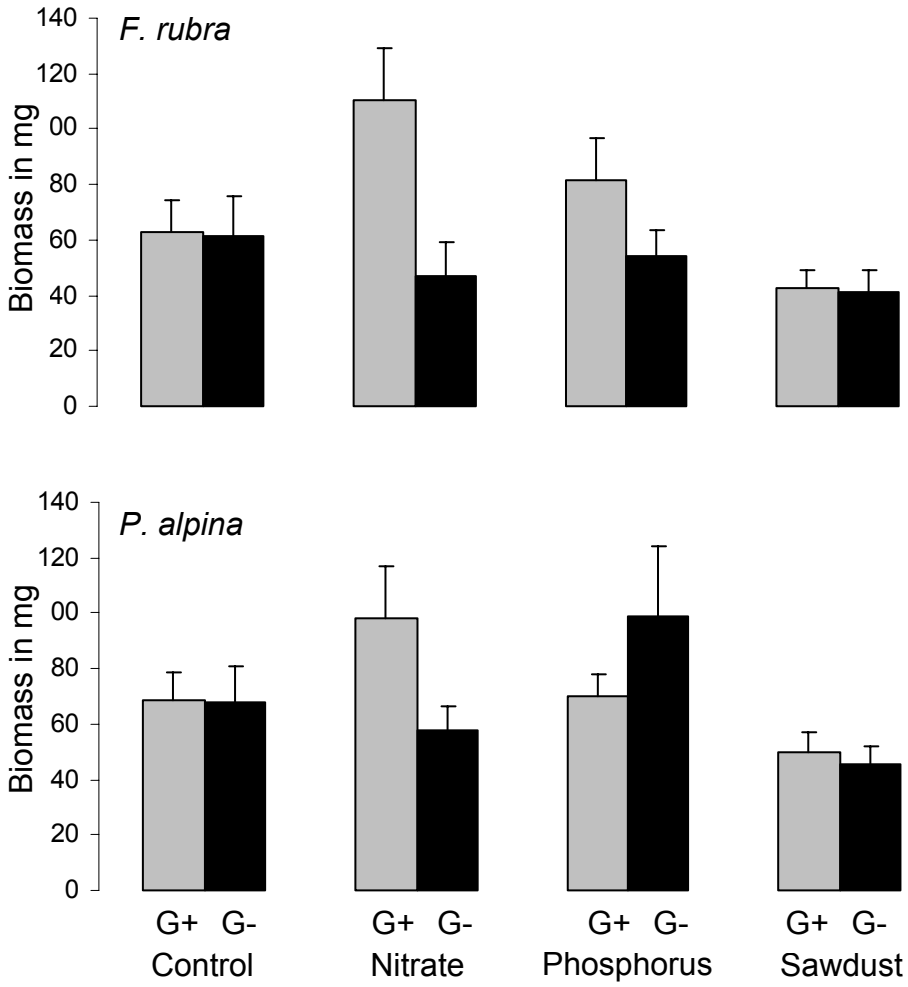


Fig. 5-3: Mean biomass in mg (+SE) of *F. rubra* (above) and *P. alpina* (below) phytometer plants per nutrient manipulation treatment and grazing treatment (G+, grazed; G-, ungrazed). For statistics see Table 5-1.

In contrast to *F. rubra*, biomass of *P. alpina* was significantly influenced by the interaction between grazing management and field nutrient manipulations (cf. Table 5-1, Fig. 5-3). While plants growing on soil from ungrazed and grazed plots had similar above-ground biomass, when subject to sawdust amendments or on control, plants growing on soil from grazed and N-amended plots did produce nearly twice as much biomass as plants from ungrazed sites. This response was opposite to that of P-fertilised subplots, where plants growing on soil form ungrazed plots did produce about 30 % more biomass.

Discussion

Nutrient manipulations, soil nutrients and above-ground biomass

The soil amendment in the field had a significant effect on the above-ground biomass of the two phytometer species under greenhouse conditions. As predicted, C-addition did have a negative effect on above-ground productivity. The effect of C-addition therefore persisted for at least 4 months, since the last sawdust treatment was applied in the field in June 2005 and soil for the greenhouse experiment sampled in October 2005. Several field studies have shown that biomass or cover of grasses tends to be more strongly reduced by C- addition than the biomass or cover of forbs (Blumenthal et al. 2003, Corbin and D'Antonio 2004, Eschen et al. 2006a). Similarly, cover of grasses, but not cover of forbs was reduced by three years of sawdust addition in the field study on mountain grasslands from which the soil samples for the present study were taken (chapter 4). This reduction may lead to reduced above-ground competition for light and increased below-ground competition for nutrients as experiments have shown that shoot:root ratio of grasses is stronger affected by C-addition than that of forbs, which had similar losses in total biomass after C-addition (Schmidt et al. 1997b, Eschen et al. 2006b). In grasslands, where competition for light is important (Tilman 1988), a reduction in shoot:root ratio of grasses after C-addition is therefore likely to improve the relative competitive ability of forbs.

Both phytometer plants reached a higher biomass on grazed sites when N-fertilised suggestion N-limitation at grazed sites (cf. Fig. 5-1).

Grazing has shown to enhance the mineralization of nutrients compared to ungrazed sites (McNaughton et al. 1997, Zeller et al. 2000), and to increase the N-concentration in living biomass of grasses, leading to a decrease in plant available nutrient over time (Chaneton et al. 1996), as biomass is removed by cattle which redistribute nutrients unevenly by dunging. As a consequence litter quantity and quality is altered and soil microbial community changes from fungi-dominant at grazed sites to bacteria-dominant at abandoned sites (Bardgett et al. 1997). As the quality and quantity of C supplied by plants through root exudates and root turnover can stimulate the microbial activity and determine the rate of net N-mineralization (Schmidt et al. 1997b), grazing effects nutrient cycling. In accordance with these mechanisms, both species were N-limited under grazed conditions.

While the above-ground biomass was significantly affected by the nutrient manipulation treatments applied in the field, no differences were found in the soil nutrient concentration. Several reasons may explain this phenomenon. Maximal plant nutrient uptake on mountain grasslands occurs normally immediately after snowmelt when nutrients are released from the microbial biomass. However, our soil samples were taken in late autumn, where N-content measured as nitrate or ammonium content was already low. Secondly, in arctic and alpine ecosystems with low amounts of plant available N, plants and microbes probably compete primarily for N at the organic-monomer stage (Schimel and Bennett 2004). With the soil analysis methods chosen, only the inorganic compounds of the total plant available N pool, namely nitrate and ammonium, were measured while amino-acids and a large part of the in the organic soil matter fixed N is not

considered. However, as in cold climate ecosystems plant can also access the organic N soil pool, we used a high AMF-colonized and a lower colonized phytometer species to investigate on the importance of this direct N-pathway between soil organic matter or amino-acids and plants.

Responses of *F. rubra* and *P. alpina* to benomyl application

In accordance with our predictions, above-ground biomass of *P. alpina* was not affected by the benomyl treatment. *P. alpina* had a mycorrhizal infestation rate by about 75 % lower compared to *F. rubra*. *P. alpina* can therefore only minimally profit from inorganic N or fixed P due to the nearly absence of AMF (only 4 % of the roots were infested by AMF), and it must rely on the nitrate and ammonium for its N supply and on soluble P for its P-supply. Two line of evidence support this view. First, the productivity of *P. alpina* indicated N limitation on grazed sites and P-limitation on ungrazed sites (cf. Fig. 5-3). Secondly, the higher N:P-ratio on ungrazed sites compared to grazed sites leads to the same conclusion that grazed sites were N limited and ungrazed sites restricted by P availability.

Effects of benomyl and nutrient treatments on *F. rubra*

In contrast the non-mycorrhizal plant *P. alpina*, the AMF-plant *F. rubra* was significantly affected by the nutrient manipulation x benomyl interaction. Benomyl addition lead to a higher productivity of *F. rubra* on P-amended plots, while biomass on sawdust amended soils was lower when benomyl was applied.

On previously C-amended soil, *F. rubra* plants yielded a lower biomass when benomyl was applied. *F. rubra* was in our study well infested by AMF on control plots what facilitates the access to organic N for the plant (Lipson and Monson 1998, McKane et al. 2002, Nordin et al. 2004). As microbes compete in many cases better for nutrients compared to plants (Schmidt et al. 1997b), and C-additions stimulate microbial activity, sawdust reduces the inorganic N content in the soil (Schmidt et al. 1997a). Decreasing the AMF infestation rate through benomyl application harms or interrupts the direct access to organic N what is reflected in the lower biomass production of *F. rubra*. The lower above-ground biomass of *F. rubra* is the result of a lower inorganic soil nutrient status (through C-addition) and the restricted potential to acquire organic N via the AMF pathway. As predicted, this directed path is interrupted through benomyl or in the absence of AMF as observed with *P. alpina* which was not affected by the benomyl treatments.

Much more tricky to interpret are the observed changes on the P-amended soil due to the benomyl treatment. On soils where we applied benomyl, we observed a nearly one third higher biomass yield of *F. rubra* compared to biomass of plants growing on untreated soil. Biomass of *F. rubra* plants on P-amended, benomyl treated soils was only minimally lower than that from the mycorrhized control plants on N fertilised soil (86.3 mg vs. 88.9 mg). One possible explanation could be that the release of large amounts of polyphosphates stored in the fungal vacuoles (Ferrol et al. 2002). This would argue for high nutrient immobilisation by AMF in mountain grassland soils similar to arctic soils where up to 30 % of total P is stored it the microbial biomass (Jonasson et al. 1999). But in contrast to a similar study where the

authors sterilised their soil by autoclavation prior to the in the greenhouse applied nutrient manipulation treatments (Schmidt et al. 1997a), we did not observe a general increase in productivity due to the benomyl application. Autoclavation is know to increase the inorganic nutrients by release of nutrients from killed microbial populations (Troelstra et al. 2001). This was also the case for Schmidt's et al. study, where after sterilization the ammonium concentration increased 13 times and phosphate increased 33 times. Application of benomyl had on the other hand only little effect on the soil nutrient concentration (Kahiluoto et al. 2000 , but see below). This could explain, why an increase in the productivity in our study is only observed in the P amended soil, where high P availability due to the fertilisation allowed AMF to store P, which was released after killing the AMF.

Conclusions

Our greenhouse study provides evidence that depending on land-use form N or P can be the limiting resource on mountain grasslands, and that C-addition is a promising tool to reduce productivity. Nevertheless AMF-plants are less affected by the treatments as the symbiosis with mycorrhizae allows them to directly access organic N. Non-AMF-plants which rely on inorganic compounds must struggle for those with soil microorganisms and are therefore likely to be more dependent on soil inorganic nutrients. As microbes compete at least temporally in many cases better for nutrients compared to plants, and C-additions stimulates microbial activity (Schmidt et al. 1999), sawdust additions should reduce the plant available N content in the soil. C-additions may thus play a critical role in the control of the nutrient

cycling process, in particular for grasses which have a low mycorrhizal dependency (van der Heijden 2002)

Acknowledgements

This work was funded by the Swiss Priority Program (NFP 48) “Landscapes and Habitats of the Alps”, a research project of the Swiss National Science Foundation (grant 4048-064424 to US).

References

Alpert, P. and Maron, J. L. 2000. Carbon addition as a countermeasure against biological invasion by plants. - Biol. Invasions 2: 33-40.

Bardgett, R. D., Leemans, D. K., Cook, R. and Hobbs, P. J. 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. - Soil Biol. Biochem. 29: 1285-1294.

Blaschke, H. 1991. Distribution, mycorrhizal infection, and structure of roots of calcicole floral elements at treeline, Bavarian Alps, Germany. - Arctic, Antarctic, and Alpine Research 23: 444-450.

Blumenthal, D. M., Jordan, N. R. and Russelle, M. P. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. - Ecol. Appl. 13: 605-615.

Chaneton, E. J., Lemcoff, J. H. and Lavado, R. S. 1996. Nitrogen and phosphorus cycling in grazed and ungrazed plots in a temperate subhumid grassland in Argentina. - J. Appl. Ecol. 33: 291-302.

Corbin, J. D. and D'Antonio, C. M. 2004. Can carbon addition increase competitiveness of native grasses? A case study from California. - Restor. Ecol. 12: 36-43.

Eschen, R., Mortimer, S. R., Lawson, C. S., Edwards, A. R., Brook, A. J., Igual, J. M., Hedlund, K. and Schaffner, U. 2006a. Carbon addition alters early succession on ex-arable fields. -: submitted.

Eschen, R., Müller Schärer, H. and Schaffner, U. 2006b. Soil carbon addition affects plant growth in a species-specific way. - J. Appl. Ecol.: in press.

Ferrol, N., Barea , J. M. and Azcón-Aguilar, C. 2002. Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas. - Plant Soil 244: 231–237.

Fitter, A. H. and Nichols, R. 1988. The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. - New Phytol. 110: 201-206.

Fontaine, S., Bardoux, G., Abbadie, L. and Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. - Ecol. Lett. 7: pp. 314-320.

Frank, D. A., Gehring, C. A., Machut, L. and Phillips, M. 2003. Soil community composition and the regulation of grazed temperate grassland. - Oecologia 442: 603-609.

Gough, L., Osenberg, C. W., Gross, K. L. and Collins, S. L. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. - Oikos 89: 428-439.

Gross, K. L., Willig, M. R., Gough, L., Inouye, R. and Cox, S. B. 2000. Patterns of species density and productivity at different spatial scales in herbaceous plant communities. - Oikos 89: 417-427.

Hartnett, D. C. and Wilson, G. W. T. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. - Plant Soil 244: 319-331.

Jonasson, S., Michelsen, A., Schmidt, I. K. and Nielsen, E. V. 1999. Responses in microbes and plants to changed temperature, nutrient and light regimes in the Arctic. - Ecology.

Jonasson, S., Michelsen, A., Schmidt, I. K., Nielsen, E. V. and Callaghan, T. V. 1996. Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. - Oecologia 106: 507-515.

Kahiluoto, H., Ketoja, E. and Vestberg, M. 2000. Creation of a non-mycorrhizal control for a bioassay of AM effectiveness - 1. Comparison of methods. - Mycorrhiza 9: 241-258.

Kahmen, S., Poschlod, P. and Schreiber, K. F. 2002. Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. - Biol. Conserv. 104: 319-328.

Knops, J. M. H. and Tilman, D. 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. - Ecology 8: 88-98.

Lipson, D. A. and Monson, R. 1998. Plant–microbe competition for soil amino acids in the alpine tundra: effects of freeze–thaw and dry–rewet events. - Oecologia 113: 406–414.

McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., Giblin, A. E., Kielland, K., Kwiatkowski, B. L., Laundre, J. A. and Murray, G. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. - Nature 415: 68-71.

McLendon, T. and Redente, E. F. 1992. Effects on nitrogen limitation on species replacement dynamics during early secondary succession on a semiarid sagebrush site. - Oecologia 91: 312-317.

McNaughton, S. J., Banyikwa, F. F. and McNaughton, M. M. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. - Science 278: 1798-1800.

Michelsen, A., Graglia, E., Schmidt, I. K., Jonasson, S., Sleep, D. and Quarmby, C. 1999. Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. - New Phytol. 143: 523-538.

Nordin, A., Schmidt, I. K. and Shaver, G. R. 2004. Nitrogen uptake by artic soil microbes and plants in relation to soil nitrogen supply. - Ecology 85: 955-962.

Paul, N. D., Ayres, P. G. and Wyness, L. E. 1989. On the use of fungicides for experimentation in natural vegetation. - Funct. Ecol. 3: 759-769.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. - Transactions British Mycological Society 55: 158-161.

Read, D. J. and Haselwandter, K. 1981. Observations on the mycorrhizal status of some alpine plant communities. - New Phytol. 88: 341-352.

Rillig, M. C. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. - Ecol. Lett. 7: 740-754.

Sala, O. E., Chapin III, F. S., Armesto, J. J., Berlow, E., Bloomfeld, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A. P., Leemans, R., Lodge, D. M., Mooney, H. A.,

Oesterheld, M., Poff, L., Sykes, M. T., Walker, B. H., Walker, M. and Wall, D. H. 2000. Global biodiversity scenarios for the year 2100. - Science 287: 1770-1774.

Schimel, J. P. and Bennett, J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. - Ecology 85: 591-602.

Schmidt, I. K., Jonasson, S. and Michelsen, A. 1999. Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. - Applied Soil Ecology 11: 147-160.

Schmidt, I. K., Michelsen, A. and Jonasson, S. 1997a. Effect on plant production after addition of labile carbon to arctic/alpine soils. - Oecologia 112: 305-313.

Schmidt, I. K., Michelsen, A. and Jonasson, S. 1997b. Effects of labile soil carbon on nutrient partitioning between an arctic graminoid and microbes. - Oecologia 112: 557-565.

Schmidt, I. K., Ruess, L., Bååth, E., Michelsen, A., Ekelund, F. and Jonasson, S. 2000. Long-term manipulation of the microbes and microfauna of two subarctic heaths by addition of fungicide, bactericide, carbon and fertilizer. - Soil Biol. Biochem. 32: 707-720.

Smith, M. D., Hartnett, D. C. and Rice, C. W. 2000. Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. - Soil Biol. Biochem. 32: 935–946.

Suding, K. N., Collins, S. L., Gough, L., Clark, C., Cleland, E. E., Gross, K. L., Milchunas, D. G. and Pennings, S. 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. - Proc. Nat. Acad. Sci. USA 102: 4387-4392.

The R Development Core Team. 2003. R 1.8.1.

Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities. - University Press Princeton.

Török, K., Szili-Kovács, T., Halassy, M., Tóth, T., Hayek, Z., Paschke, M. W. and Wardell, L. J. 2000. Immobilization of soil nitrogen as a possible method for the restoration of sandy grassland. - Appl. Veg. Sci. 3: 7-14.

Troelstra, S. R., Wagenaar, R., Smant, W. and Peters, B. A. M. 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. - New Phytol. 150: 697–706.

Tscherko, D., Hammesfahr, U., Marx, M.-C. and Kandeler, E. 2004. Shifts in rhizosphere microbial communities and enzyme activity of *Poa alpina* across an alpine chronosequence. - Soil Biol. Biochem. 36: 1685–1698.

van der Heijden, M. G. A. 2002. Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search of underlying mechanisms and general principles. - In: Sanders, I. R. (ed.) Mycorrizhal ecology. Springer, pp. 243-265.

van der Heijden, M. G. A. 2004. Arbuscular mycorrhizal fungi as support systems seedling establishment in grassland. - Ecol. Lett.: 293-303.

Van der Putten, W. H., Vet, L. E. M., Harvey, J. A. and Wäckers, F. L. 2001. Linking above and belowground multitrophic interactions of plants, herbivores, pathogens and antagonists. - Trends Ecol. Evol. 16: 547-551.

West, H. M., Fitter, A. H. and Watkinson, A. R. 1993. The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata ssp. ambigua* under natural conditions. - J. Ecol. 81: 345-350.

Zeller, V., Bahn, M., Aichner, M. and Tappeiner, U. 2000. Impact of land-use change on nitrogen mineralization in subalpine grasslands in the Southern Alps. - Biol. Fertility Soils 31: 441-448.

Synthesis

In the following, I try to connect the results obtained in the each study and link them with observations in accompanying studies and literature. Two points are of particular interest: changes in land use and their consequences for species richness and unpalatable weeds, and the effects of sawdust addition on nitrogen cycling.

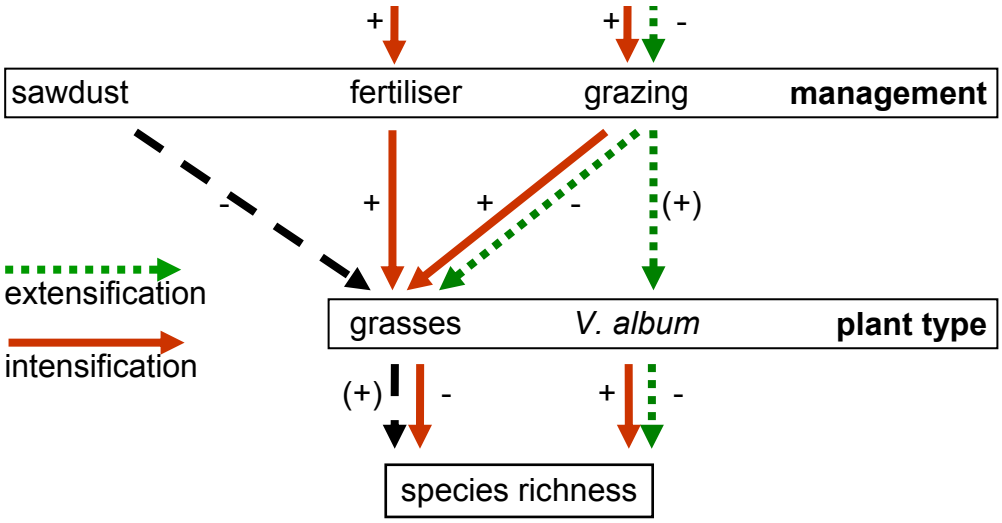
Land use changes and plant species richness

Theory predicts that in particular in ecosystems with a long grazing history like mountain grasslands, decreasing traditional grazing densities leads to a reduced plant species diversity (Milchunas et al. 1988). Increasing productivity above a critical threshold also decrease the diversity of grasslands (Mittelbach et al. 2001). An important mechanism for these two patterns is competition. Some authors suggest that competition for above-ground resources (light) may be greater where biomass production is high, but that competition for below-ground resources (nutrients) is greater where biomass is low (Grime 1973, Newman 1973, Tilman 1986). Similar patterns were found in our studies.

When traditionally managed pastures are extensified (green arrows in Fig. 6-1), grazing pressures is gradually reduced until complete abandonment. As biomass is no longer removed, plants grow bigger what increases above-ground competition for light (Newman 1973). Grasses are fast-growing and possess plant traits allowing them to maximise nutrient uptake, in contrast to slower growing forbs which have developed mechanisms to optimise nutrient uptake (Schippers and Olff 2000). Grasses can therefore profit more

from a gradually abandonment of grazing compared to forbs and become more abundant. After some time, biomass will accumulate what reduces mineralization rate (Zeller et al. 2000). In consequence, limitation of plant available nutrients increases, shifting competition from above- to below-ground. Now slower growing forbs may win head-to-head competition situations. The abandoned grasslands surveyed in chapter 2, were at the state where grasses dominate the vegetation. At this point, abandonment of grazing does not have a significant influence on species richness (cf. chapter 2), but resulted in a loss of uncommon or rare plant species characteristic for traditional pastures and in an establishment of forest species. This change will result in reduced plant species richness in the future. This decrease in species richness may be accelerated by the presence of *V. album*. On abandoned sites we found a negative relationship between species richness and cover of *V. album*. Moreover, studies in mountain grasslands have shown that unpalatable plants such as *V. album* can facilitate the establishment of late-successional woody species (Smit 2005).

Fig. 6-1: Influence of management options on species richness of mountain grasslands for extensification and intensification scenarios.



Intensification (red arrows in Fig. 6-1) of mountain grasslands increases the grazing intensity, but also the nutrient availability through fertiliser application. Grasses have a high grazing tolerance (WallisDeVries et al. 1998), and are able to rapidly recover after grazing events. They have developed traits to rapidly take up high quantities of plant available nutrient (see above). Both mechanisms help grasses to become more abundant on fertilised and intensively grazed pastures compared to traditionally used grasslands (chapter 2). However, grasses are correlated to species richness in a second order polynomial way (cf. Fig. 6-2). On the left side of a threshold value of about 40 % grass cover, vegetation is dominated by tall and dense forbs (*megaphorbiae*). Those vegetational associations are in general species poor and have low grass cover. From 40 % grass cover onwards, species richness declines with increasing grass cover indicating a negative effect of high grass cover on species richness.

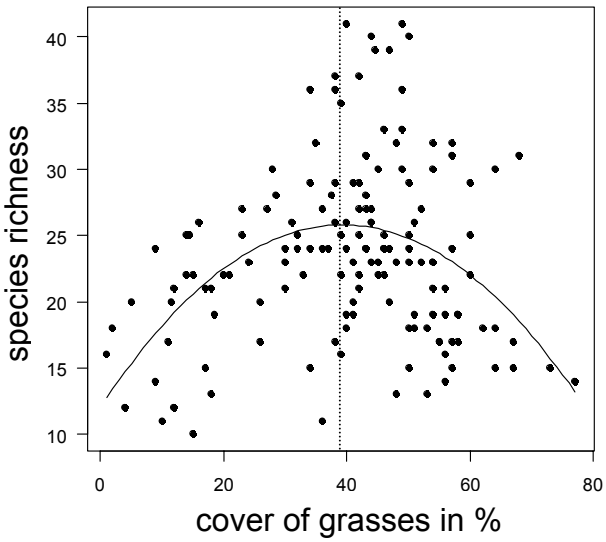


Fig. 6-2: The relationship between the species richness in 1 m² plots and the cover of grasses on mountain grasslands in the Alps. Vertical dashed line indicates maximum of the curve (r^2 =0.18, p <0.001).

Sawdust addition has shown to reduce in particular the cover of grasses (chapter 4). It could therefore be a method to mitigate the negative impact of an increased grass cover. Contrary to grasses, *V. album* promotes on small spatial scales species richness on

intensively used pastures indication facilitation of grazing sensitive plant species by the unpalatable weed.

Nitrogen cycling and sawdust addition

Nutrient availability is one of the major determinants of above-ground productivity of plants, and nitrogen (N) the limiting factor in many terrestrial ecosystems (Vitousek et al. 1997). Fig. 6-3a shows the mechanics of soil N cycling in mountain grasslands in a schematic way. Plants produce litter which is incorporated by soil macro-organisms into the soil organic matter. In cold climate ecosystems like mountain grasslands, where litter input is low and decomposition and N cycling are slow, plants and microbes compete primarily for N at the organic stage i.e. the amino-acids (Schimel and Bennett 2004). Microbes are relatively N limited (Schimel and Chapin 1996), but some of the N would be mineralised to ammonium, and, if nitrifiers are present, small part will also be reduced to nitrate (at our study sites the ammonium:nitrate ratio was about 10:1). As carbon additions

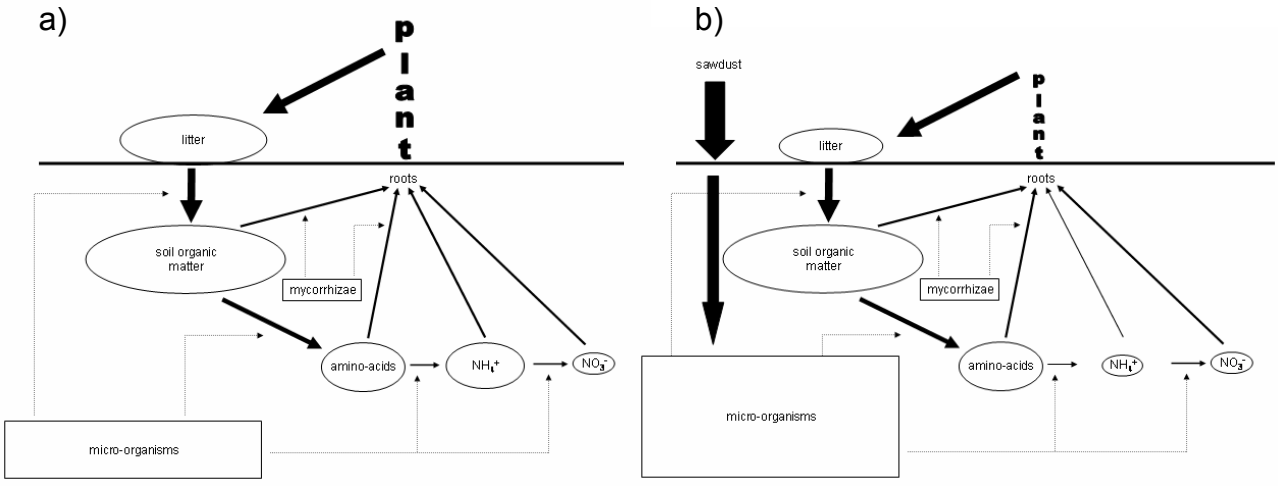


Fig. 6-3: Nitrogen fluxes in a grasslands ecosystem without (a) or with additional carbon (b). Solid lines indicate nitrogen fluxes, dashed lines show where micro-organisms interact. Mycorrhizae are part of the soil micro-organisms, but are for a better understanding represented separately. Only pathways which are important for the here described mechanism are shown.

stimulates microbial activity (Blumenthal et al. 2003), and microbes compete in many cases better for nutrients compared to plants (Schmidt et al. 1999), sawdust additions (cf. Fig. 6-2b) should reduce the inorganic nitrogen content in the soil (Corbin and D'Antonio 2004). Indeed, in a short-time experiment on one of our study sites, addition of rapidly available sugar decreased the soil ammonium content significantly, and also the leaf N-content of *Dactylis glomerata* and *Leontodon hispidus* (Spiegelberger et al., unpubl. data).

In alpine ecosystems, acquisition of nitrogen with the aid of arbuscular mycorrhizal fungi (AMF) directly from the soil organic matter may be more important than in lowland ecosystems. The majority of herbaceous plants in arctic and alpine ecosystems form symbiotic relationships with AMF (Read and Haselwandter 1981, Blaschke 1991) and a therefore up to a certain level, independent of inorganic nitrogen. Results from the greenhouse study (chapter 5) indicated that AMF play an important role in the N-uptake. Application of the fungicide benomyl which decreases the biomass of AMF (Fitter 1986, Merryweather and Fitter 1996), reduced the above-ground biomass of the highly AMF-infested plant *Festuca rubra* in sawdust-amended soil, but did not influence the above-ground biomass of the less AMF-infested *Poa alpina*. AMF facilitate the direct access to organic nitrogen for the plant (Lipson and Monson 1998, McKane et al. 2002, Nordin et al. 2004). Decreasing the AMF infestation rate through benomyl application harms or interrupts the direct access to organic nitrogen which may explain the lower biomass production of *F. rubra*.

An open question is how carbon addition affects nutrient cycling in the long-term. It is possible that the soil nutrients temporally fixed in

the soil micro-organisms are not completely released, but that a new balance between soil nutrient, soil microbes and plants is created. Addition of sawdust is quite likely not only to change the activity of microbes, but similar to results of lime addition (chapter 3), also the composition of the microbial community. Decreased productivity and in the long-term perhaps a changed vegetation composition, affects, through differences among species and in the quality and the quantity of resources input to the soil, both the soil microbial composition and the magnitude of microbial processes (Wardle 2002 and references therein). Microbial species that are able to decompose the modified litter can increase in quantity and be more dominant in the altered microbial community. Feedbacks between the above- and belowground communities could then maintain the induced changes (van der Heijden and Cornelissen 2002, Wardle and van der Putten 2002).

Sawdust addition seems to have the potential to increase in the long-term species richness of mountain grasslands, as it reduced in particular the dominance of grasses, and may create gaps in the dense vegetation cover allowing germination and establishment of new plants. Species richness of mountain grasslands seems to be micro-site limited, as artificially created gaps in natural vegetation of mountain grassland let to a significant increase in seedlings recruitment (Spiegelberger et al., unpub. results). However, establishment of new species may be limited by seed availability, as seed addition lead during the same experiment to an increase in seedling recruitment.

Management implications

Based on the present work, we recommend maintaining traditional land use of mountain grasslands where this is feasible. Traditionally used, extensively grazed pasture have shown to harbour the highest species diversity compared to intensively used pastures and abandoned grasslands. *V. album* has no measurable impact on species richness on traditionally used pastures, but can favour species richness on intensively used pastures. These results can probably be extended to other large and unpalatable species similar like *Gentina lutea* or *Crisium eriophorum*. They should therefore be preserved as patches of unpalatable plants may serve as starting points in the creation of species-rich islands within a pasture.

On pastures which have been fertilised in the past and where the cover of grasses is high, sawdust addition could be an easily practicable, low-cost solution to alter vegetation composition in a desirable way. The experiments of this study demonstrated that in particular the dominant functional group of grasses is over-proportionally harmed by the treatment (chapter 4). Grasses are - when present in high abundance - negatively related to species richness on mountain grasslands (see above). In consequence, reducing the cover of grasses could be a promising method to maintain or may increase species richness of mountain grasslands. However, whether short-time application of sawdust may have a long-lasting effect on the biodiversity of mountain grasslands remains an open question.

Responsible management with regard to the high biodiversity of mountain grasslands should include a sustainable control of the soil pH. Soil pH is known to be a key factor concerning plant diversity (Pärtel 2002) and substantially influences soil microbial communities (Bååth et al. 1995, Bååth and Anderson 2003), probably through plant-soil microbial feedbacks (Wardle and van der Putten 2002). As shown in chapter 3, changes in soil pH have important consequences on vegetation composition.

Conclusion

This work has generated new valuable insights in the interrelationship between land use, soil nutrient availability, and biodiversity on mountain grasslands. It has shown how long-lasting land use change can affect mountain grassland ecosystems and is among the first which provides evidence that sawdust addition has the potential to reduce soil nutrient availability and thus alter vegetation of mountain grasslands in a desirable way. On small spatial scale species unpalatable weeds influence species richness, but the outcome depends on land use: unpalatable weeds are positively related to species richness suggesting facilitation on fertilised, intensively grazed pastures, and negatively on abandoned grasslands indicating competition for space. However, at large spatial scales, their role for biodiversity is less important, but when present in high abundance, they are likely to speed up the process of a gradual abandonment of traditionally used grasslands.

References

Bååth, E. and Anderson, T. H. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. - Soil Biol. Biochem. 35: 955-963.

Bååth, E., Frostegård, A., Pennanen, T. and Fritze, H. 1995. Microbial community structure and pH response in relation to soil organic-matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. - Soil Biol. Biochem. 27: 229-240.

Blaschke, H. 1991. Distribution, mycorrhizal infection, and structure of roots of calcicole floral elements at treeline, Bavarian Alps, Germany. - Arctic, Antarctic, and Alpine Research 23: 444-450.

Blumenthal, D. M., Jordan, N. R. and Russelle, M. P. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. - Ecol. Appl. 13: 605-615.

Corbin, J. D. and D'Antonio, C. M. 2004. Can carbon addition increase competitiveness of native grasses? A case study from California. - Restor. Ecol. 12: 36-43.

Fitter, A. H. 1986. Effect of Benomyl on leaf phosphorus concentration in alpine grassland: a test of mycorrhizal benefit. - New Phytol. 103: 767–776.

Grime, J. P. 1973. Competitive exclusion in herbaceous vegetation. - Nature 242: 344–347.

Lipson, D. A. and Monson, R. 1998. Plant–microbe competition for soil amino acids in the alpine tundra: effects of freeze–thaw and dry–rewet events. - Oecologia 113: 406–414.

McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, E. B., Fry, B., Giblin, A. E., Kielland, K., Kwiatkowski, B. L., Laundre, J. A. and Murray, G. 2002. Resource-based niches

provide a basis for plant species diversity and dominance in arctic tundra. - Nature 415: 68-71.

Merryweather, J. and Fitter, A. H. 1996. Phosphorus nutrition of an obligately mycorrhizal plant treated with the fungicide benomyl in the field. - New Phytol. 132: 307–311.

Milchunas, D. G., Sala, O. E. and Lauenroth, W. K. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. - Am. Nat. 132: 87-106.

Mittelbach, G. G., Steiner, C. F., Scheiner, S. M., Gross, K. L., Reynolds, H. L., Waide, R. B., Willig, M. R., Dodson, S. I. and Gough, L. 2001. What is the observed relationship between species richness and productivity? - Ecology 82: 2381-2396.

Newman, E. I. 1973. Competition and diversity in herbaceous vegetation. - Nature 244: 310-311.

Nordin, A., Schmidt, I. K. and Shaver, G. R. 2004. Nitrogen uptake by artic soil microbes and plants in relation to soil nitrogen supply. - Ecology 85: 955-962.

Pärtel, M. 2002. Local plant diversity patterns and evolutionary history at the regional scale. - Ecology 83: 2361-2366.

Read, D. J. and Haselwandter, K. 1981. Observations on the mycorrhizal status of some alpine plant communities. - New Phytol. 88: 341-352.

Schimel, J. P. and Bennett, J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. - Ecology 85: 591-602.

Schimel, J. P. and Chapin, F. S. 1996. Tundra plant uptake of amino acid and NH_4^+ nitrogen in situ: Plants compete well for amino acid N. - Ecology 77: 2142–2147.

Schippers, P. and Olff, H. 2000. Biomass partitioning, architecture and turnover of six herbaceous species from habitats with different nutrient supply. - Plant Ecol. 149: 219-231.

Schmidt, I. K., Jonasson, S. and Michelsen, A. 1999. Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. - Applied Soil Ecology 11: 147-160.

Smit, C. 2005. Facilitation of tree regeneration in wood pastures. Department of Ecology and Evolution. - University of Fribourg, pp. 8-96.

Tilman, D. 1986. The resource-ratio hypothesis of plant succession. - Am. Nat. 125: 827-852.

van der Heijden, M. G. A. and Cornelissen, J. H. C. 2002. The critical role of plant-microbe interactions on biodiversity and ecosystem functioning: arbuscular mycorrhizal associations as an example. - In: Loreau, M., Naeem, S. and Inchausti, P. (eds.), Biodiversity and ecosystem functioning - synthesis and perspectives. Oxford University Press, pp. 181-192.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H. and Tilman, D. G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. - Ecol. Appl. 7: 737-750.

WallisDeVries, M. F., Bakker, J. P. and Van Wieren, S. F. (eds.). 1998. Grazing and Conservation Management. - Kluwer Academic Publishers.

Wardle, D. A. 2002. Communities and ecosystems - linking the aboveground and belowground components. - Princeton University Press.

Wardle, D. A. and van der Putten, W. H. 2002. Biodiversity, ecosystem functioning and above-ground-below-ground linkages. - In: Loreau, M., Naeem, S. and Inchausti, P. (eds.), Biodiversity and ecosystem functioning - synthesis and perspectives. Oxford University Press, pp. 155-168.

Zeller, V., Bahn, M., Aichner, M. and Tappeiner, U. 2000. Impact of land-use change on nitrogen mineralization in subalpine grasslands in the Southern Alps. - Biol. Fertility Soils 31: 441-448.

Acknowledgments

After having carried 1900 kg of sawdust to the field and 800 kg soil from the field, having climbed about twice the Mt. Everest (up AND down), I am at the point where I should thank my legs, arms and back, but also the people who helped during this time.

I wish to express my thanks to Urs whose door is always open (expect if Hariet is around…). You helped very much in the beginning, but probably even more in the end with critical comments and revisions of numerous drafts after you kids went to bed or during daily train trips. Thank you very much, Urs, I learned a lot! Thanks also to Heinz for giving me the opportunity to do my thesis on a fascinating topic, and in the Alps. Your comments and suggestions have been very valuable.

Diethart is thanked for being the external referee for this thesis, and for his patience in answering question about study design, analyses and statistics, and also for his critical comments and useful suggesting during writing the manuscripts and this thesis. Thanks to Katarina Hedlund for teaching me how to do PLFA analysis and other lab work (which I started to enjoy at least a little bit …).

Thanks to Tom Steinlein for the good cooperation with his students Julia Binkenstein, Claudia Hogekamp, Michael Pühra and Jasmin Weisshäuptl.

Marie-Pierre Bazan, Ghislaine Cortat, René Eschen, Marius Grecu, Stefan Gross, Elze Hesse, Heather Kirk, Jason Knight, Christophe Praz, Chris Smit, Jakob Troxler, and Travis Turner assisted with practical work and logistical help.

Mauro Bassignana, André Borand, Nicolas Doutaz, Philippe Mestelan helped finding field sites (probably the hardest part of the study…), and thanks to all the land-owners who allowed working on their fields.

… and last but not least, thanks to all the people at CABI.

Curriculum vitae - Thomas Spiegelberger

Born 28th December 1973 in Munich, Germany.

Education

2002 - 2006	University of Fribourg, Switzerland. PhD. in biology "Land use, soil nutrient availability and conservation of biodiversity on mountain grasslands"
1998 - 1999	ENITHP (Ecole Nationale d'Ingénieurs des Travaux de l'Horticulture et du Paysage), Angers, France
1996 - 2002	Technical University of Munich, department of landscape architecture and ecology, Freising-Weihenstephan, Germany
1993 - 1995	Applied training as landscape gardener, Kirchdorf, Germany
1984 - 1995	Schyren-Gymnasium, D- 85276 Pfaffenhofen/Ilm, Germany

Professional career

since 2006	Research Scientist, CEMAGREF Grenoble, France
2002 - 2006	Research Scientist, CABI Bioscience, Delémont, Switzerland
2001	Scientific collaborator, KWATA, Cayenne, French Guiana
1999 - 2001	Research assistant, TU München, Freising-Weihenstephan, Germany.
1999	Junior landscape engineer, Branch Landscape Associates, Malvern, Worcestershire, England.

Articles in international peer-review journals

- Spiegelberger, T., Matthies, D., Müller-Schärer, H., Schaffner, U. (2006) Sawdust addition as a promising tool to reduce productivity of nitrogen enriched mountain grasslands. Submitted.
- Spiegelberger, T., Hegg, O., Matthies, D., Hedlund, K., Schaffner, U. (2006) Long-term effects of short-term perturbation in a sub-alpine grassland. Ecology, 87, pp. 1939-1944
- Spiegelberger, T., Matthies, D., Müller-Schärer, H., Schaffner, U. (2006) Scale-dependent effects of land use on plant species richness of mountain grassland in the European Alps. Ecography, 29, pp. 541-548
- Spiegelberger, T. and Ganslosser, U. (2005) Food habitats of the Antillean manatees (*Trichechus manatus manatus*) in the Coswine Swamps (French Guiana, South America). Tropical Zoology 18. pp. 1-12.
- de Thoisy, B., Spiegelberger, T., Rousseau, S., Talvy, G., Vogel , I. and Vié, J.-C. (2003) Distribution, habitat, and conservation status of the West Indian Manatee *Trichechus manatus* in French Guiana. - Oryx 37(4). pp. 431-436.
- Sliva, J., Spiegelberger, T. and Buchhart, M. (2000) Renaturierung von Feuchtgebieten Bayerns – erste Ergebnisse einer Umfrage. TELMA 30. pp.125-130.

Oral and poster presentations

- Spiegelberger, T., Hegg, O., Matthies, D., Hedlund, K., Schaffner, U. (2005) Long-term effects of short-term perturbation on a sub-alpine grassland. Oral presentation, International Botanical Congress, Vienna, Austria.
- Spiegelberger, T., Schaffner, U. and Müller-Schärer, H. (2005) Plant species richness on mountain grasslands changes with land-use and spatial scale. Poster presentation, Biology 05 symposium, Basel, Switzerland.
- Spiegelberger, T. (2005) La flore alpine. Oral presentation, Club alpine français, Belfort, France.
- Spiegelberger, T., Schaffner, U. and Müller-Schärer, H. (2004) Spatial patterns of plant species richness on mountain grasslands altered by changed land-use practise. Oral presentation, 34rd GfÖ annual meeting, Giesen, Germany.

- Müller-Schärer, H., Schaffner, U., Hesse, E., Spiegelberger, T. (2004) Veratrum album in moutain grasslands under changing farming activities. NFP Workshop, Eggiwil, Switzerland.
- Spiegelberger, T. (2004) Le Vératre et la richesse floristique sur les pâturages de montagne. Oral presentation, Departmental Seminar, University of Grenoble, France.
- Spiegelberger, T. (2004) Can sawdust addition reduce weed pressure on mountain grasslands? Oral presentation, Research Seminar, University of Fribourg.
- Spiegelberger, T. (2003) Species richness and productivity. Oral presentation, Interaction Seminar, University of Fribourg.
- Spiegelberger, T., Schaffner, U. and Müller-Schärer, H. (2003) Nitrogen reduction via carbon addition on mountain pastures. Poster presentation, BES annual meeting, Lancaster, UK.
- Spiegelberger, T., Schaffner, U. and Müller-Schärer, H. (2003) Changes in patterns of plants species richness and unpalatable plants from intensive mountain pastures to abandoned grasslands. Poster presentation, 33rd GfÖ annual meeting, Halle, Germany.
- Spiegelberger, T., Schaffner, U. and Müller-Schärer, H. (2003) Does a gradual abandonment of traditional management favour the spread of weeds and loss of plant species diversity on mountain grassland? Poster presentation, International meeting of the French section of IALE, Gap, France.
- Spiegelberger, T., Schaffner, U. and Müller-Schärer, H. (2003) Plant species richness and unpalatable plants over different spatial scales and management methods on mountain pastures. Oral presentation, NCCR Symposium Biodiversity & Species Interaction, Neuchâtel, Switzerland.

Other publications

- Spiegelberger, T. (2007) L'ambiguïté d'une « mauvaise » herbe. Espaces naturels, in press.
- Spiegelberger, T., Murell, C. and Gerber, E. (2005) Inventaire des sites et des surfaces occupés par les renouées et par l'impatiente glanduleuse sur la commune de Delémont. Unpublished report, CABI Bioscience. 12 pp.
- Müller-Schärer, H., Schaffner, U., Hesse, E., Spiegelberger, T. (2004) Suche nach einem Unkrautmanagement für Alpweiden. Montagna 15, pp. 15-17.

- Spiegelberger, T. (2002): Analysis of habitats used by manatees (Trichechus manatus manatus) in the side-rivers of Maroni Estuary in French Guiana. Diploma thesis. Department of Vegetation Ecology, TU Munich. 204 pp.

Publications in preparation

- Spiegelberger, T., Schaffner, U. Nutrient manipulation on mountain grassland – the importance of arbuscular mycorrhizal fungi. In preparation for Oecologia.
- Spiegelberger T., Weishäupl, J., Steinlein, T., and Schaffner, U. Short-term effects of carbon addition on above- and belowground parameters of a mountain pastures. In preparation for Restoration Management.
- Spiegelberger, T., Steinlein, T., Schaffner, U. Seedling recruitment of mountain grasslands restricted by space and herbivory. In preparation for Journal of Ecology.
- Spiegelberger, T., Stohlgren, T., Schaffner, U. Comparison of species richness of alpine pastures in the Rocky Mountains and the Alps. In preparation for Ecological Monographs.
- Eschen, R. and T. Spiegelberger. Carbon addition as a tool for prairie restoration – a review. In preparation for Restoration Ecology.

Printed by CEMAGREF editions
Paris

December 2006